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FINAL REPORT
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Formation of Mutagens from the Atmospheric Photooxidants of PAH and their Occurrence in Ambient Air

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

AIR RESOURCES BOARD
Research Division

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Final Report

Contract No. A132-075

Prepared for:

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Disclaimer

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Acknowledgments

We express our appreciation to our Project Officer, Ralph Propper, for many helpful discussions and encouragement throughout this entire program. Dr. José López-Cancio is thanked for his contributions to the ambient analyses conducted during his stay at SAPRC as a visiting scientist. We thank Ms. Sara M. Aschmann for very able technical assistance during the environmental chamber experiments and Ms. Susan I. Waddy for assistance in the fiscal management of this program. A very special thanks is due to Ms. Christy J. LaClaire for typing and assembling this report.

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Abstract

Ambient air analyses demonstrated that the polar mutagenic reaction product of the simulated atmospheric photooxidation of phenanthrene, 2-nitrodibenzopyranone, is a wide-spread contaminant in the environment. 2-Nitrodibenzopyranone was observed in all of the approximately two dozen southern California ambient air filter and polyurethane foam (PUF) plug samples analyzed, and 4-nitrodibenzopyranone (also an atmospheric reaction product of phenanthrene) was observed in the majority of these samples. Both isomers were observed in the Standard Reference Material (SRM) 1649 urban dust.

The results obtained from our laboratory experimental studies (which continued the work of our previous ARB Contract No. A732-154) showed that simulated atmospheric photooxidations of the gas-phase 2- to 4-ring polycyclic aromatic hydrocarbons (PAH) lead to mutagenic products. The specific PAH studied were: 1-methylnaphthalene, 2-methylnaphthalene, acenaphthene, acenaphthylene, biphenyl, dibenzothiophene, anthracene, retene (1-methyl-7-isopropylphenanthrene), fluoranthene, pyrene, benz[*a*]anthracene and chrysene. The total mutagenic activities of the product mixtures from the simulated atmospheric reactions of the PAH varied widely depending on the particular PAH, and also the mutagenicity profile varied from PAH to PAH, at least in terms of the polarity of the mutagenic products. In general, the PAH studied lead to either mutagenic nitro-PAH which elute, with the HPLC fractionation program used here, in fraction #3 or 4, or to more polar products including the nitro-PAH lactones. Our data, when compared with ambient air mutagenicity testing using the same sample collection, extraction, fractionation and bioassay testing procedures, allow ~50% of ambient air direct-acting mutagenicity (gas plus particle phase), *Salmonella typhimurium* strain TA98 (-S9, Kado microsuspension modification of Ames assay), to be ascribed to PAH atmospheric transformation products formed in the atmosphere during transport from source to receptor. Our data also allow a ranking of the PAH studied to be made with respect to the number of revertants per unit PAH concentration ("mutagenicity formation potential"), with a range of over three orders of magnitude from biphenyl and benz[*a*]anthracene (the lowest) to fluoranthene (the highest). It is possible that this ranking can be used in assessments of emission changes brought about through emission control measures or the use of alternate fuels.

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INTRODUCTION

Diesel exhaust is being reviewed as a toxic air contaminant by the California Air Resources Board (ARB), with the gas- and particle-phase polycyclic aromatic hydrocarbons (PAH) and PAH-derivatives being diesel exhaust constituents of particular concern. The carcinogen, benzo[a]pyrene, and the PAHs as a compound class have also been nominated for review as toxic air contaminants. The PAH are formed in combustion systems at high temperatures (Bockhorn *et al.*, 1982; Kittleson *et al.*, 1985; Prado *et al.*, 1985, Toqan *et al.*, 1985; Frenklach *et al.*, 1988; Frenklach, 1989), and hence are emitted from vehicle exhausts as well as other combustion sources, including industrial processes, domestic and commercial heating systems, waste incineration facilities, tobacco smoking, agricultural burning, and several natural processes such as forest fires and volcanic eruptions (Nikolaou *et al.*, 1984). Many of the PAH are animal carcinogens (NAS, 1983) and they are mutagenic in the presence of microsomal activation (+S9) in the Ames *Salmonella typhimurium* bacterial assay (McCann *et al.*, 1975).

In the atmosphere, the PAH are distributed between the gas and particle phases (see, for example, Arey *et al.*, 1987; Atkinson *et al.*, 1988; Bidleman, 1988; Coutant *et al.*, 1988). Theoretical and ambient air data for several series of semi-volatile organic compounds (including PAH, alkanes, and organochlorine compounds) show that organic compounds with liquid-phase vapor pressures $> 10^{-6}$ Torr exist in the atmosphere at least partially in the gas phase (Bidleman, 1988). Recent ambient atmospheric measurements of the PAH in California's atmosphere using high-volume samplers equipped with Teflon-impregnated glass fiber (TIGF) filters for the particulate matter and polyurethane foam (PUF) solid adsorbent for the semi-volatiles show that the 2- to 4-ring PAH exist mainly in the gas phase (Arey *et al.*, 1987, 1989a; Atkinson *et al.*, 1988), although it must be recognized that the gas/particle distributions derived from adsorbent samplers using filter-solid adsorbent combinations are "operational" phase distributions and may be subject to a number of artifact problems (Bidleman, 1988).

A. Evidence for Atmospheric Transformations of Gas-Phase PAH

A recent ARB-funded ambient air measurement study provided clear evidence for the atmospheric reactions of the volatile PAH with the hydroxyl (OH) radical, with the nighttime/daytime concentration ratios exhibiting a clear linear correlation with the OH radical reaction rate constant (Figure 1) [Arey *et al.*, 1989a]. From an estimate of the nighttime dilution rate, provided by the daytime/nighttime ratio of 3-nitrobiphenyl [a nitroarene believed to be formed only in the atmosphere from the daytime reaction of biphenyl with the OH radical in the presence of NO_x (Atkinson *et al.*, 1987)], an average 12-hr daytime OH radical concentration of 2.2×10^6 molecule cm^{-3} (during August) was derived, uncertain to at least a factor of 2. This estimated OH radical concentration in an urban area is essentially identical to the annually averaged global tropospheric 12-hr daytime OH radical concentration of 1.5×10^6 molecule cm^{-3} (Prinn *et al.*, 1987) and provides strong evidence that the gas-phase PAH do react in the troposphere. Further evidence is provided, as discussed below, by the nitro-PAH observed in ambient atmospheres.

B. Formation of Nitro-PAH and Other PAH-Derivatives

Laboratory studies carried out over the past ten years at the Statewide Air Pollution Research Center (SAPRC), University of California, Riverside, have shown that the 2- to 4-ring PAH present in the gas-phase undergo reactions in the atmosphere, initiated by the OH radical, to form nitroarenes, with the nitroarenes being distributed between the gas and particle phases and the 4-ring nitrofluoranthenes and nitropyrenes being in the particle phase in the atmosphere (Sweetman *et al.*, 1986; Arey *et al.*, 1986, 1989a,b, 1990; Atkinson *et al.*, 1987, 1990a,b; Atkinson and Aschmann, 1988). Indeed, the particle-phase nitroarenes observed in ambient air arise primarily from the atmospheric transformations of fluoranthene and pyrene (Arey *et al.*, 1986, 1990; Zielinska *et al.*, 1988, 1989a; Atkinson *et al.*, 1990a). As demonstrated by the gas chromatography/mass spectrometry (GC/MS) data shown in Figure 2, 2-nitrofluoranthene and 2-nitropyrene are the major nitro-PAH formed from the OH radical-initiated reactions of fluoranthene and pyrene, respectively. 1-Nitropyrene, the electrophilic nitration product of pyrene, is emitted into the atmosphere in diesel exhaust and other direct emissions (Schuetzle

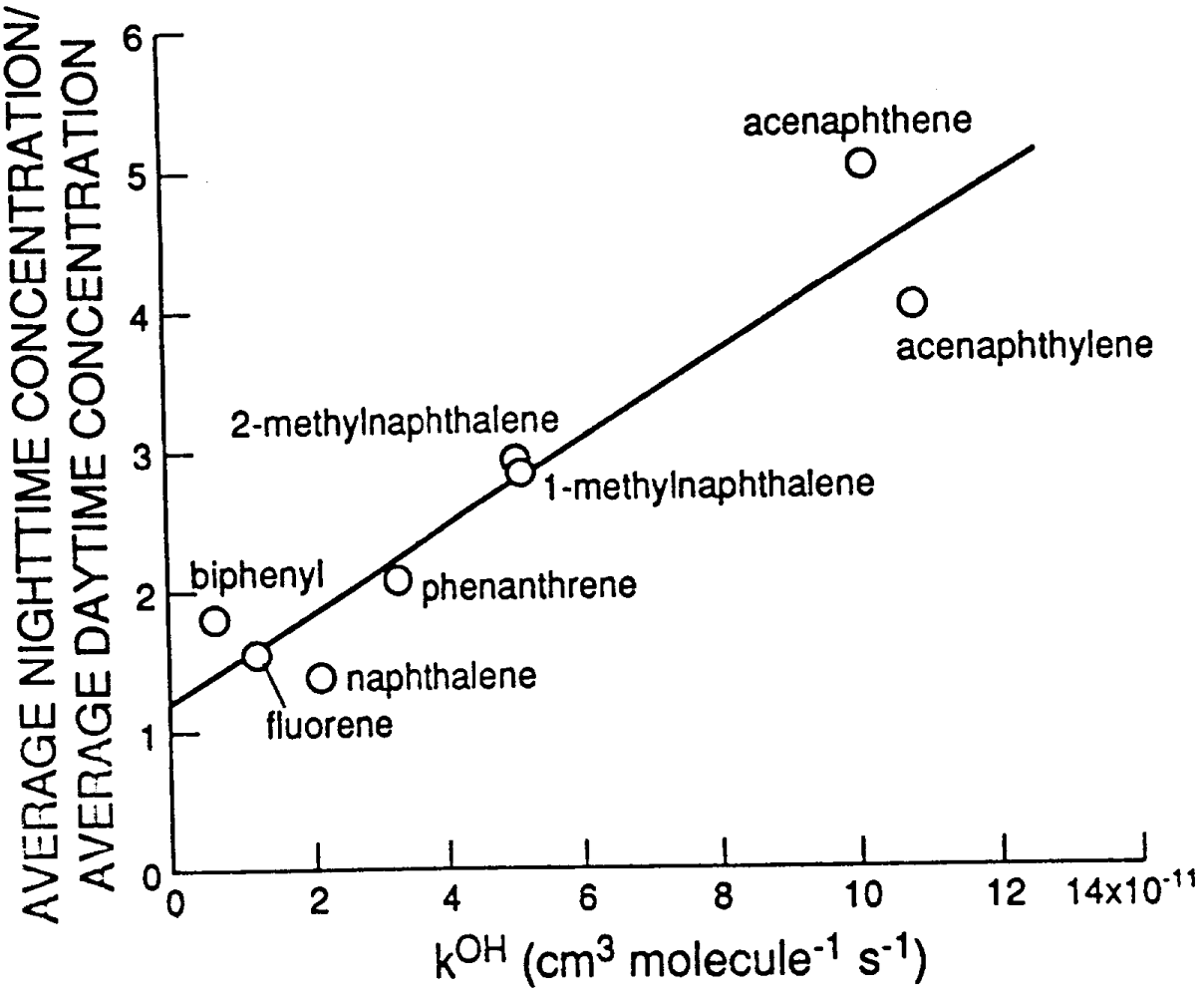


Figure 1. Plot of the average nighttime/average daytime concentration ratio for the volatile PAH measured in Glendora, CA against their OH radical reaction rate constants (adapted from Arey *et al.*, 1989a).

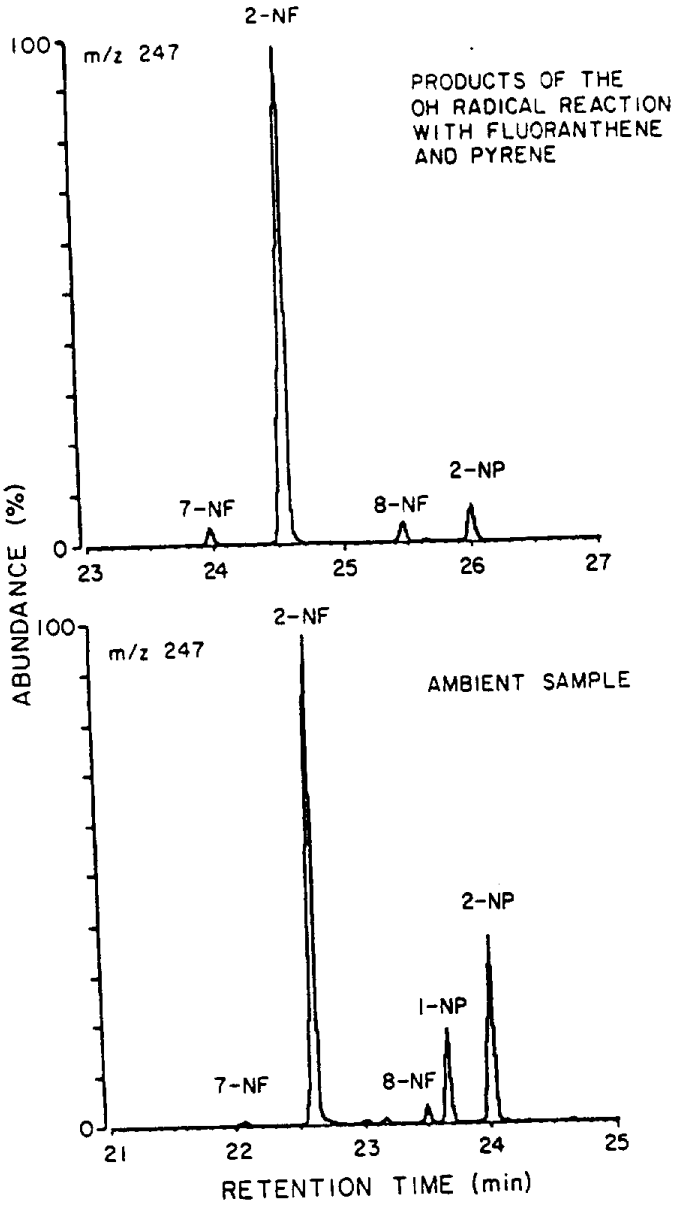


Figure 2. GC/MS analyses of: a sample collected from a chamber exposure of fluoranthene and pyrene to OH radicals in the presence of NO_x (top) and a daytime ambient air sample collected at Torrance, CA, during a high-NO_x episode (bottom). Shown are the molecular ions m/z 247 for the nitrofluoranthenes (NF) and nitropyrenes (NP). The same column phase (5% phenylmethylsilicone) was used for both sample analyses, however, they were conducted at different times and the column and/or conditions were different. The separation is expected to be very similar and the elution order identical for the two analyses, although the retention times are obviously not identical.

et al., 1981; Gibson, 1983; Paputa-Peck *et al.*, 1983; Schuetzle, 1983; Harris *et al.*, 1984) and is observed in the ambient sample shown in Figure 2. More abundant than the 1-nitropyrene in the ambient sample, however, are 2-nitrofluoranthene and 2-nitropyrene, nitro-PAH not reported in direct emissions. We have analyzed ambient particles from many locations in the United States and in Europe and 2-nitrofluoranthene has always been more abundant than 1-nitropyrene in these samples (Pitts *et al.*, 1985; Arey *et al.*, 1986; Ramdahl *et al.*, 1986; Sweetman *et al.*, 1986; Zielinska *et al.*, 1989a; Atkinson *et al.*, 1988).

In an analogous manner, the majority of the gas-phase 2-ring nitroarenes, 1- and 2-nitronaphthalene, methylnitronaphthalenes and 3-nitrobiphenyl, also appear to arise from the gas-phase reactions of the naphthalene, 1- and 2-methylnaphthalene and biphenyl precursors (Atkinson *et al.*, 1987; Arey *et al.*, 1987, 1989a, 1990; Zielinska *et al.*, 1989b). Using the measured ambient concentrations of the PAH, their OH radical reaction rate constants, their nitroarene formation yields and the photolysis rates for the nitronaphthalenes, we were able to predict ambient nitronaphthalene, 3-nitrobiphenyl, 2-nitrofluoranthene and 2-nitropyrene concentrations in good agreement with those measured (Arey *et al.*, 1990). Thus, we now have a reasonable understanding of the atmospheric loss processes of the gaseous two- to four-ring PAH and the formation and loss processes of the two- to four-ring nitroarenes.

The formation yields of the nitroarenes from the OH radical-initiated reactions of the 2- to 4-ring PAH are $\sim 5\%$ in all cases (Arey *et al.*, 1989b; Atkinson *et al.*, 1990a), with most of the remaining products presently being unaccounted for [hydroxy-PAH have been observed from the OH radical-initiated reactions of naphthalene and biphenyl with yields of $\sim 10\%$ and $\sim 20\%$, respectively (Atkinson *et al.*, 1987)].

C. Ambient Mutagenicity

It has been recognized for many years that extracts of collected ambient respirable particulate matter are carcinogenic (Leiter *et al.*, 1942) and it has recently been shown that these extracts are strongly mutagenic in the Ames assay without microsomal activation [i.e., they are direct-acting mutagens] (Pitts *et al.*, 1977, 1982; Talcott and Wei, 1977; Tokiwa *et al.*, 1977). This direct-acting mutagenicity cannot be due to the PAH, since the PAH require microsomal activation. To date, the chemical compounds responsible for the direct-acting mutagenicity of

collected ambient particulate matter have not been determined to any appreciable extent. Nitroarenes, many of which are strong direct-acting mutagens and animal carcinogens (Rosenkranz and Mermelstein, 1983; IARC, 1984; Tokiwa and Ohnishi, 1986; King, 1988), have been identified and quantified in ambient particulate matter collected at several locations throughout the world (see, for example, Gibson, 1983; Nielsen, 1983; Tokiwa *et al.*, 1983; Nielsen *et al.*, 1984; Pitts *et al.*, 1985; Nielsen and Ramdahl, 1986; Ramdahl *et al.*, 1986; Sweetman *et al.*, 1986; Arey *et al.*, 1987, 1988; Atkinson *et al.*, 1988; Zielinska *et al.*, 1988, 1989a). However, measurements of the nitroarenes present in ambient particulate matter and of the mutagenicity of extracts of the ambient particulate matter show that the nitroarenes contribute typically 1-10% of the measured mutagenicity (Atkinson *et al.*, 1988; Arey *et al.*, 1988; Strandell *et al.*, 1987), with 2-nitrofluoranthene and 2-nitropyrene generally being the most abundant particle-phase nitroarenes and (together with 8-nitrofluoranthene in many cases) the major contributors to ambient particulate mutagenicity (Arey *et al.*, 1988; Atkinson *et al.*, 1988).

Although the nitroarenes (mostly produced *in situ* in the atmosphere by reactions of the gas-phase 2- to 4-ring PAH) contribute $<10\%$ of ambient particle-phase direct-acting mutagenicity, the direct-acting mutagenicity of extracts of collected ambient air particulate matter at seven sites in California impacted by differing combustion emissions gave a reasonable correlation with the 2-nitropyrene concentrations (Atkinson *et al.*, 1988) but not with the measured PAH concentrations. Since 2-nitropyrene is formed in the atmosphere from the OH radical-initiated reaction of pyrene, this observation suggests that the bulk of the direct-acting mutagenicity in ambient air particulate matter arises from the gas-phase atmospheric reactions of the 2- to 4-ring PAH (Atkinson *et al.*, 1988). That the mutagenic profile of primary combustion-generated emissions undergoes change during transport from source to receptor is shown by the data given in Figure 3 (Pitts *et al.*, 1984). The mutagrams [bar graphs of mutagenic activity *versus* high performance liquid chromatography (HPLC) fraction number (fraction polarity)] in Figure 3 shows that while a major portion of the direct-acting mutagenicity of a diesel exhaust extract is present in a fraction containing the nitroarenes (fraction #7 under the analysis conditions shown in Figure 3), this is not the most mutagenically potent fraction of the ambient particulate matter.

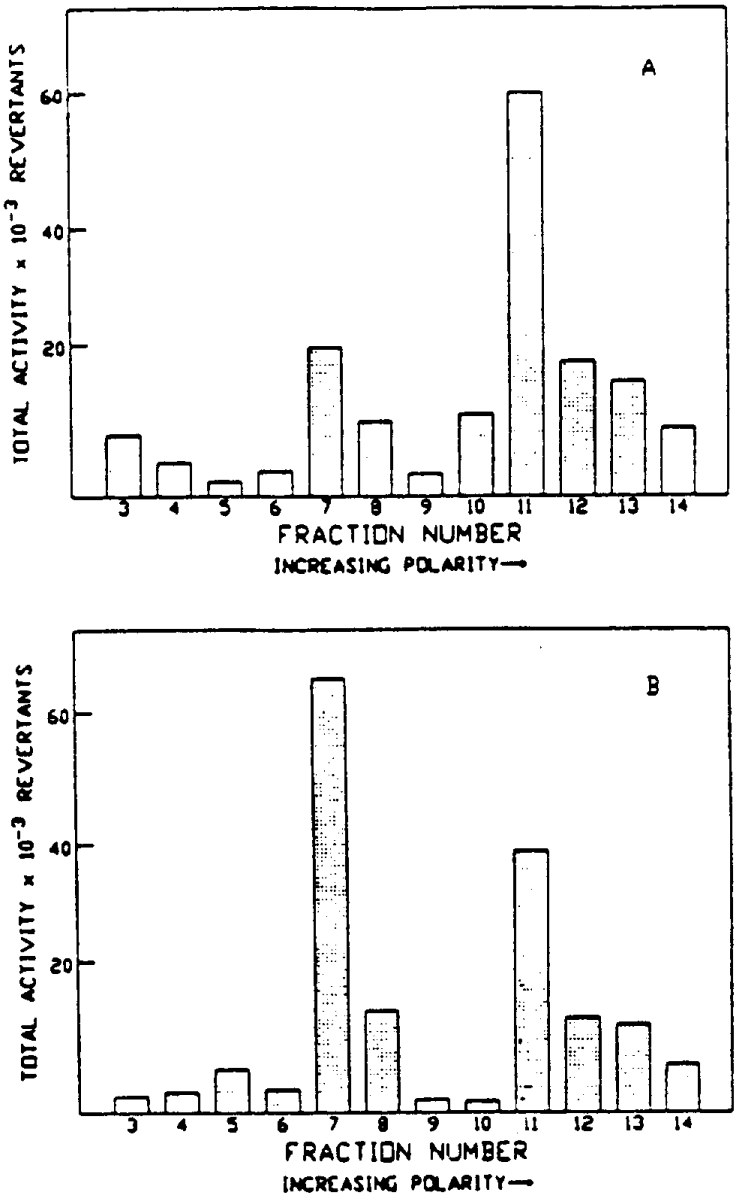


Figure 3. Mutagrams showing the mutagenic activities (TA98, -S9) of HPLC fractions of CH_2Cl_2 extracts of (A) an ambient POM sample collected in El Monte, CA, and (B) a diesel exhaust particulate sample.

Thus, as shown in Figure 3, the mutagenicity of the ambient particulate matter extract occurred mainly in the more polar fraction #11. These findings have since been confirmed by ourselves (Atkinson *et al.*, 1991) and others (Siak *et al.*, 1985; Schuetzle and Lewtas, 1986, Lewtas *et al.*, 1990).

D. Identification of Mutagenic PAH Transformation Products

The observations that the PAH undergo atmospheric transformations to form nitroarenes and other reaction products, and that the nitroarenes contribute $< 10\%$ of ambient particle-phase mutagenicity, but correlate with the ambient particle-phase mutagenicity, suggest that the majority of ambient mutagenicity, presently not accounted for, is due to PAH atmospheric reaction products. Moreover, the mutagenicity polarity profile of ambient air extracts show that the majority of the mutagenicity is due to the presence in ambient air particulate matter of chemical compounds more polar than the nitroarenes. Using bioassay-directed fractionation of the products from gas-phase reactions of selected PAH in our environmental chamber, we recently identified (under ARB Contract No. A732-154, completed 5/31/91) a new class of compounds, the nitro-PAH lactones, which may contribute significantly to the mutagenic activity of ambient particulate extracts (Atkinson *et al.*, 1991).

In this previous ARB-funded contract (A732-154), we conducted hydroxyl (OH) radical-initiated reactions of the PAH naphthalene, fluorene and phenanthrene (among the most abundant PAH in ambient atmospheres [see, for example, Atkinson *et al.*, 1988]) in our 6400 liter all-Teflon chamber, using the photolysis of methyl nitrite (CH_3ONO) to generate OH radicals at concentrations over an order of magnitude higher than present in the ambient atmosphere. After the reaction, the chamber volume was collected on polyurethane foam plugs for solvent extraction and fractionation by HPLC. Mutagenicity testing of these HPLC fractions was carried out using the Kado microsuspension modification (Kado *et al.*, 1983, 1986) of the standard Ames *Salmonella typhimurium* microbial assay, and representative mutagrams are shown in Figure 4 for naphthalene, fluorene and phenanthrene and for an extract of ambient air particulate matter collected in the Los Angeles air basin. (Note that the HPLC fraction numbers given on Figure 4 do not correspond to those of Figure 3, but in both cases polarity increases with increasing fraction number.)

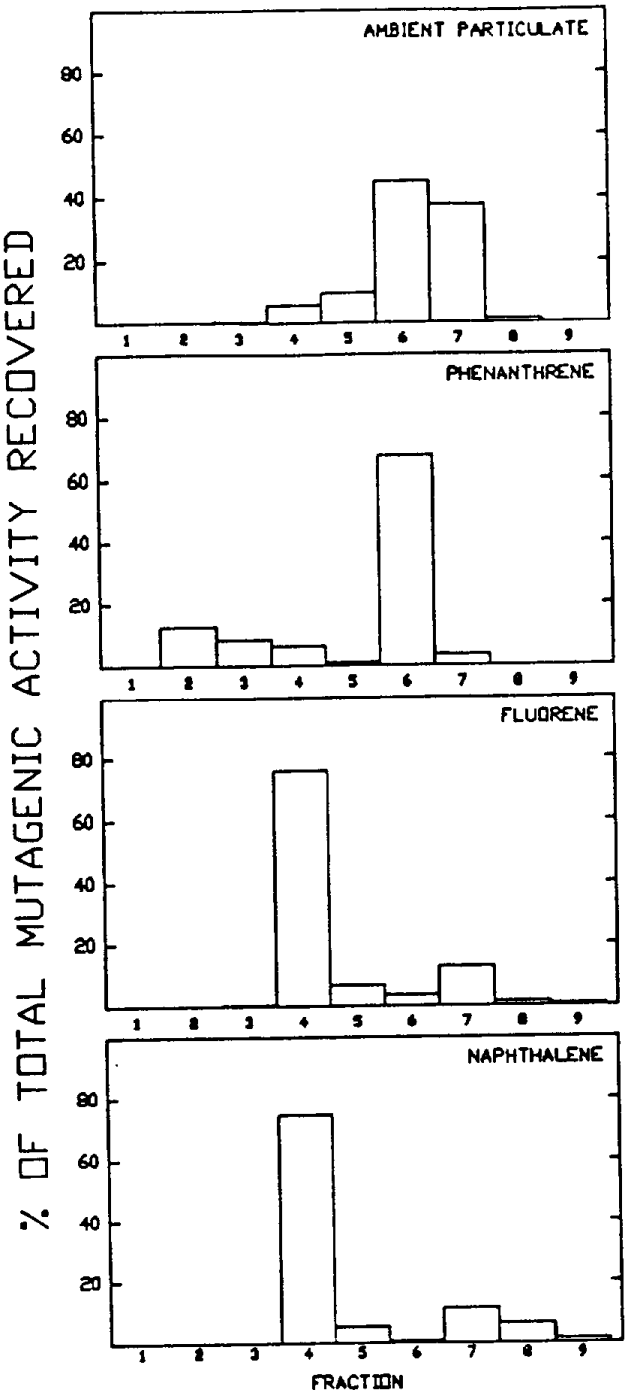
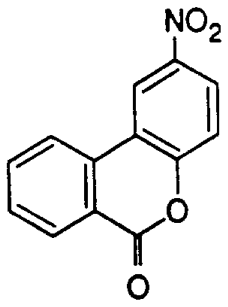


Figure 4. HPLC mutagrams from the gas-phase OH radical-initiated reactions of phenanthrene, fluorene and naphthalene compared to the mutagram of Claremont, CA ambient air particulate extracts (adapted from Arey et al., 1992). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total revertants recovered from the phenanthrene, fluorene and naphthalene chamber reactions were 341,000, 150,000, and 97,000, respectively. The fraction numbers do not correspond to those given in Figure 3.

Chemical analysis was conducted on the most mutagenic fractions. For naphthalene and fluorene, the most mutagenic fractions (fraction #4 in each case) were those containing the nitronaphthalenes and nitrofluorenes, respectively, which accounted for the majority of the measured mutagenicities in these nitroarene fractions. For phenanthrene, the mutagenicity profile resembled that of collected ambient air particulate matter, with the majority of the mutagenicity being in fraction #6, a fraction more polar than the nitroarene-containing fraction. Chemical analysis showed the presence in this mutagenic fraction of two nitrophenanthrene lactones (Arey et al., 1992). Through mutagenicity testing of available standard materials, it was determined that one isomer, 2-nitro-6H-dibenzo[b,d]pyran-6-one (I), is a highly potent, direct-acting mutagen.

(I)



E. Current Contract Work

We ended our previous ARB contract report (A732-154) by describing the GC/MS analysis of highly mutagenic HPLC subfractions from ambient particles collected in Riverside, CA, which showed the presence of the two nitrophenanthrene lactones which we had observed from our chamber experiments with phenanthrene (Helmig et al., 1992a). Also present in these mutagenic subfractions were five compounds tentatively identified as nitro-methylphenanthrene (or methylanthracene) lactones and two compounds which may be nitropyrene (or nitrofluoranthene) lactones. One element of significant effort resulting from our present contract and reported here is the quantification of 2- and 4-nitro-6*H*-dibenzo[*b*,*d*]pyran-6-one in ambient particles from southern California and in the National Institute of Standards and Technology

(NIST) standard urban dust sample (SRM 1649). As will be reported below, we also investigated the formation yields of the nitrophenanthrene lactones under various experimental conditions in an attempt to better understand the chemistry involved (Helmig et al., 1992b).

As noted above, the use of the Kado microsuspension modification of the Ames assay resulted in significant mutagenic activity from reaction products such as 2-nitronaphthalene (which accounts for the majority of the activity of fraction #4 of the naphthalene reaction products shown in Figure 4), which are expected to be present in the gas-phase under ambient conditions. This realization prompted us to investigate the mutagenic activity of the semi-volatile fraction of ambient air as collected on polyurethane foam plugs. Utilizing funding from the U.S. EPA, we examined the mutagenicity of HPLC-separated vapor-phase and particulate organics from ambient air. Shown in Figure 5 (taken from Harger et al., 1992) are the mutagrams for the vapor-phase and particulate phase from ambient air samples collected in Claremont, CA. Note that the vapor-phase activity is comparable to the activity on the particles, although the distribution among the HPLC fractions is significantly different. The polarity distribution of the mutagenicity of the vapor-phase ambient sample resembles the distribution seen in Figure 4 for the reaction products of naphthalene and fluorene. That is, the most mutagenic fraction of the vapor-phase sample is in the nitroarene-containing fraction #4.

We will report here the mutagrams resulting from the simulated atmospheric reactions of the following 2- to 4-ring PAH: 1- and 2- methylnaphthalene, acenaphthene, acenaphthylene, biphenyl, dibenzothiophene, 2-methylphenanthrene, anthracene, retene (1-methyl-7-isopropyl-phenanthrene), fluoranthene, pyrene, benz[a]anthracene and chrysene (see Figure 6 for structures). Chemical analyses of the mutagenic HPLC fractions will also be discussed. The contributions of atmospheric reaction products of the 2- to 4-ring PAH to ambient mutagenicity, both vapor-phase and particle-phase, will be discussed.

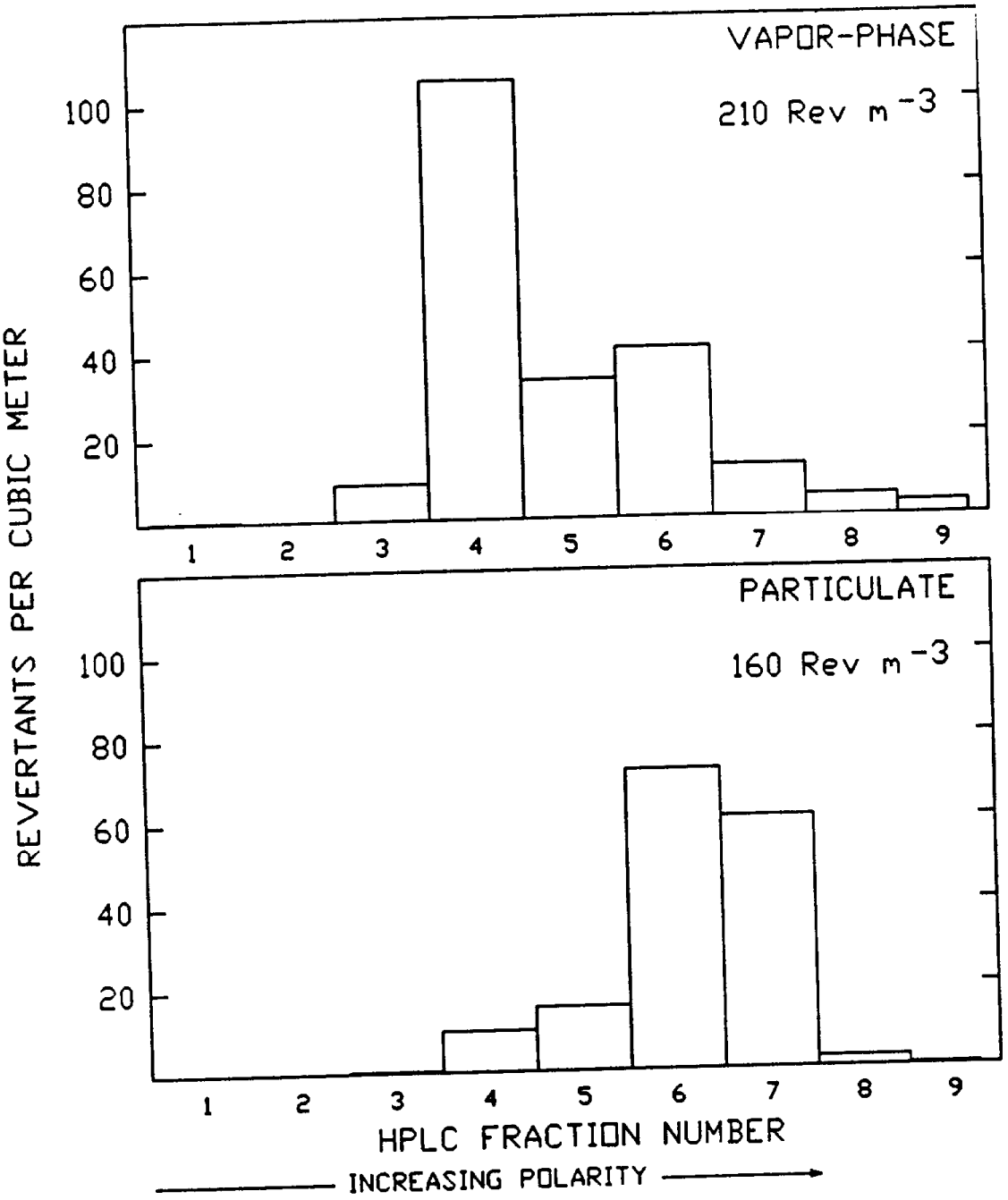
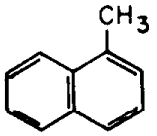
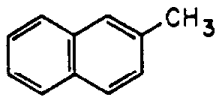


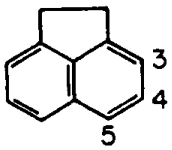
Figure 5. Mutagen density (TA98, -S9) of HPLC fractions of ambient vapor-phase and particulate organics collected on August 28, 1987 in Claremont, CA. (Adapted from Harger et al., 1992).



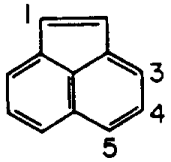
1-Methylnaphthalene



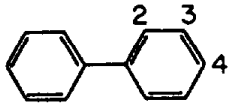
2-Methylnaphthalene



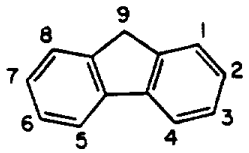
Acenaphthene



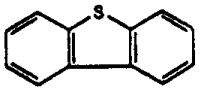
Acenaphthylene



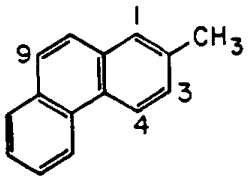
Biphenyl



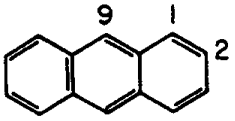
Fluorene



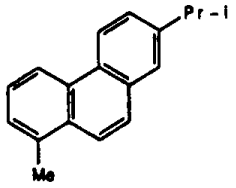
Dibenzothiophene



2-Methylphenanthrene



Anthracene



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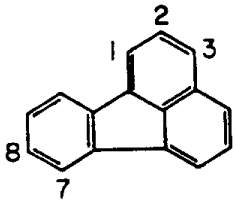
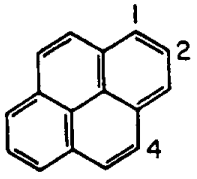
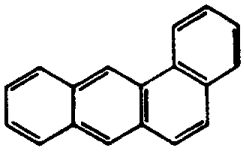


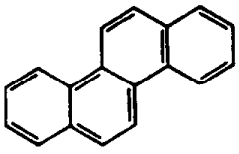
Fluoranthene



Pyrene



Benz[*a*]anthracene



Chrysene

Figure 6. Structures of the PAH studied. Naphthalene, phenanthrene (structures not shown) and fluorene were studied previously (ARB Contract No. A732-154).

MATERIALS AND METHODS

I. PHENANTHRENE: AMBIENT ANALYSES OF POLAR MUTAGENIC REACTION PRODUCTS AND MECHANISTIC STUDIES

Quantitative analyses were performed of the 2- and 4-nitrodibenzopyranones present in particle- (collected on Teflon-impregnated glass fiber filters) and gas-phase (collected on polyurethane foam plugs) samples collected in southern California. The National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs) 1649 (urban dust collected in Washington, DC) and 1650 (diesel particulate matter) were also analyzed for these PAH-derivatives. In addition, the formation of 2- and 4-nitrodibenzopyranone and related compounds from the gas-phase OH radical-initiated reactions of phenanthrene, 6H-dibenzo[b,d]pyran-6-one and 2,2'-diformylbiphenyl was investigated in environmental chamber experiments.

A. Analyses of Ambient Air Samples and Standard Reference Materials

Ambient air samples were collected using Teflon-impregnated glass fiber (TIGF) filters and polyurethane foam (PUF) plugs (Arey *et al.*, 1987; Atkinson *et al.*, 1988; Arey *et al.*, 1989a). The ambient particulate samples were collected on TIGF filters (Pallflex T60A20) using high volume samplers equipped with 10 μm size-selective inlets (General Metal Works Inc.) at calibrated flow rates of 1.1 m^3 min^{-1} (40 SCFM). These samples were collected at Claremont, CA, (Harvey Mudd College campus) and Long Beach, CA (Long Beach City College campus) during the 1987 South Coast Air Quality Study. Ambient atmospheric particulate samples were also collected at the University of California, Riverside campus in September and October, 1991, during air pollution episodes when the measured maximum daytime ozone levels were 170-230 parts-per-billion (v/v). The NIST SRM 1649 urban dust is a composite sample collected in the Washington, D.C. area over a 12-month period using a specially designed baghouse (May *et al.*, 1992). The SRM 1650 is a sample of diesel particulate material, representative of heavy-duty engine particulate emissions (May *et al.*, 1992).

The gas-phase samples were collected at Claremont during the August 1987 South Coast Air Quality Study and at Riverside during September and October 1991 on pre-extracted (dichloromethane and methanol) PUF plugs (9 cm diameter x 5 cm thickness) downstream of

TIGF filters using a high volume sampler operated at ~0.8 m^3 min^{-1} (28 SCFM). After sample collection, the filters and PUF plugs were first spiked with appropriate amounts of 3-nitrodibenzopyranone, to serve as an internal standard, and then Soxhlet-extracted with 200 mL dichloromethane for 24 h. It was confirmed from control analyses of non-spiked filter samples that this internal standard was not present at significant levels. For the SRM 1649 urban dust, 200 mg sample aliquots were Soxhlet-extracted in 2 mm cellulose thimbles (Whatman). The extracts were reduced to ~3 mL volumes with a rotary evaporator, filtered through 0.45 μm Acrodisc Teflon filters, and then reduced to ~200 μL volumes under a flow of dry nitrogen. The concentrates were separated into fractions of increasing polarity by high performance liquid chromatography (HPLC) using a semi-preparative Regis Spherisorb S5W silica (5 micron) 25 cm x 10 mm column in a Spectra-Physics Model 8100 gradient liquid chromatograph interfaced to a Model 8400 UV/Vis detector (λ = 254 nm) and ISCO fraction collector. The solvent program started with 100% hexane for 10 min, followed by a five min linear gradient to 95% hexane/5% CH_2Cl_2. The solvent was programmed over the next 25 min to 100% CH_2Cl_2 where it was held for 10 min, then programmed to 100% acetonitrile over 10 min, held isocratic for 10 min and then programmed back to the initial conditions. The flow rate was 3 mL min^{-1}. After 1 min, fractions were collected every 9 min, and fraction #6 which contained the nitrodibenzopyranones was collected in higher resolution subfractions. The quantification of the nitrodibenzopyranone isomers was achieved in the subfraction of fraction #6 covering the retention time range of 56 to 63 min. This fraction was concentrated to near dryness under a flow of dry nitrogen and then dissolved in 50 μL of dichloromethane for analysis by combined gas chromatography-mass spectrometry (GC/MS).

The NIST SRM 1650 diesel particulate material was Soxhlet extracted in 200 mg aliquots with 200 mL dichloromethane. The 3-nitrodibenzopyranone internal standard was spiked onto the thimble wall prior to the extraction. The extracts were filtered using 0.2 μm Acrodisc Teflon filter discs, concentrated to ~0.5 mL volume and prefractionated on SEP-PAK silica cartridges (Millipore) by eluting with 10 mL hexane followed by dichloromethane. The dichloromethane eluate contained the nitrodibenzopyranone isomers and was fractionated by normal-phase HPLC as described above. The nitrodibenzopyranone-containing fraction was further separated by reversed-phase HPLC on a Beckman Ultrasphere ODS 5μ column (1 cm

x 25 cm) with a Beckman Model 334 Gradient Liquid Chromatograph equipped with a Model 164 UV Detector (λ = 254 nm). The solvent used was 100% methanol, and the fraction eluting from 4 to 6 min was collected, concentrated and analyzed by GC/MS as described below.

The GC/MS analyses were carried out using a Hewlett Packard (HP) 5890 GC interfaced to an HP 5971A mass selective detector (MSD) operating in the electron impact ionization mode. The column used was a 60 m x 0.25 mm DB-1701, film thickness 0.25 μm (J&W Scientific). Injections were made on-column at 40°C oven and injector temperature, and after 2 min the oven was programmed at a rate of 25°C min^{-1} to 200°C and then at 3°C min^{-1} to 300°C. During sample injection, the column head pressure was reduced to 20 psi and then programmed at a rate of 50 psi min^{-1} to 45 psi. Detection was made in the selective ion monitoring mode (SIM), measuring the molecular ion (m/z = 241) and major fragment ions at m/z = 183 and 139 (Helmig and Arey, 1992). 2-Nitrodibenzopyranone and 4-nitrodibenzopyranone eluted with retention indices (RI) of 404.8 and 430.5, respectively [the retention indices were determined using chrysene (RI = 400) and benzo[e]pyrene (RI = 452.3) as bracketing standards (Helmig and Arey, 1992)]. The 3-nitrodibenzopyranone internal standard eluted at RI = 415.2. Quantification was made using the integrated m/z = 241 signal, and the relative response factors of the 2- and 3-nitrodibenzopyranones were determined from analyses of calibration solutions containing these isomers in varying ratios and at comparable absolute concentrations to those in the samples. The quantification of 4-nitrodibenzopyranone was carried out by assuming the same response factor as for 2-nitrodibenzopyranone.

For control blanks, pre-cleaned TIGF filters and PUF plugs were analyzed, and for the diesel analyses an empty thimble was extracted and carried through the entire sample analysis procedure. In all cases no signals with sufficient intensity for peak integration were observed. Additionally, a filter sample which had been extracted and HPLC fractionated was re-fractionated using the same HPLC conditions. It was confirmed that no significant change in the distribution of the nitrodibenzopyranones occurred during the HPLC fractionation.

B. Environmental Chamber Investigation of OH Radical-Initiated Reactions of Phenanthrene, 6H-Dibenzopyran-6-one and 2,2'-Diformylbiphenyl

A series of experiments were conducted at 298 $\pm$ 2 K and in the presence of 740 Torr total pressure of dry (<5% relative humidity) pure air to investigate the formation of 2- and 4-

nitrodibenzopyranone from the gas-phase OH radical-initiated reaction of phenanthrene in the presence of NO_x. Since the structurally-related compounds 6H-dibenzopyran-6-one and 2,2'-diformylbiphenyl were observed as products of this reaction system, their OH radical-initiated reactions were also studied. Experiments were carried out in a 6400 liter all-Teflon chamber equipped with two parallel banks of blacklamps. Because of their low vapor pressures, phenanthrene, dibenzopyranone and 2,2'-diformylbiphenyl were introduced into the chamber by spraying a methanol solution of the compound into the chamber with the chamber mixing fan (rated at 300 liter s^{-1}) on (Atkinson *et al.*, 1990a). The chamber was then flushed for ~30 min to remove the majority of the methanol.

Hydroxyl radicals were generated by the photolysis of methyl nitrite (CH_3ONO) in air at wavelengths >300 nm (Atkinson *et al.*, 1989) and NO was also added to the reactant mixtures. The initial CH_3ONO and NO concentrations were in the range (5-25) x 10^{13} molecule cm^{-3} and (2.0-24) x 10^{13} molecule cm^{-3}, respectively. Irradiations were carried out at the maximum light intensity for 1-10 min, and after the irradiation a large volume (~1000-3900 liter) gas sample was collected over 1-4 min onto two PUF plugs held in series in a glass holder. Nitric oxide was monitored by a chemiluminescence NO-NO_x monitor and gas-phase phenanthrene concentrations before and after the reactions were measured by GC with flame ionization detection (FID) by collecting 100 cm^3 gas samples onto Tenax solid adsorbent with thermal desorption at ~300 °C onto a 15 m DB-5 megabore column at 60 °C, which was then temperature programmed at 8 °C min^{-1}. The PUF plugs were analyzed for the nitrodibenzopyranones and structurally-related compounds by GC/MS employing the full scanning mode for compound identification and SIM for quantifications of the nitrodibenzopyranones, using either the HP 5890 GC and HP 5971A MSD system (see above) or a HP 5890 GC and HP 5970 MSD system with a 60 m x 0.248 mm DB-5 column (J&W Scientific) temperature programmed from 50 °C to 150 °C at 25 °C min^{-1} and then to 325 °C at 4 °C min^{-1} (with helium as the carrier gas).

C. Bioassay

Salmonella typhimurium, strain TA98, without S9 activation was used to assay the HPLC fractions of ambient air samples collected in Claremont, CA (Arey *et al.*, 1992; Harger *et al.*,

1992). To enhance sensitivity, a microsuspension preincubation modification of the Ames assay (Kado *et al.*, 1983; 1986), which employs a 90-min preincubation of the test substance with elevated test cell densities in a small-volume buffered-saline suspension was employed. The activities of 2-nitro-6H-dibenzo[b,d]pyran-6-one, as received and after HPLC purification, were the same within the reproducibility of the assay.

D. Chemicals

2-Nitro-6H-dibenzo[b,d]pyran-6-one, 3-nitro-6H-dibenzo[b,d]pyran-6-one (98% stated purity) and phenanthrene (98% stated purity) were obtained from the Aldrich Chemical Company, and 4-nitro-6H-dibenzo[b,d]pyran-6-one was synthesized as described previously (Helmig and Arey, 1992). 6H-Dibenzo[b,d]pyran-6-one was synthesized by reacting diphenic acid (Aldrich Chemical Co.) with hydrogen peroxide and sulfuric acid according to a procedure given by Ota and Okazaki (Ota and Okazaki, 1970). 2,2'-Diformylbiphenyl was obtained from the ozonolysis of phenanthrene in methanol solution (Bailey and Erickson, 1973). The identities and purities of the synthesized compounds were established by GC/MS and high resolution MS.

II. SELECTED PAH: MUTAGRAMS AND CHEMICAL ANALYSES

A. Chamber Exposures of PAH

The PAH investigated in this Contract (1-methylnaphthalene, 2-methylnaphthalene, acenaphthene, acenaphthylene, biphenyl, dibenzothiophene, anthracene, 2-methylphenanthrene, retene, fluoranthene, pyrene, benz(a)anthracene and chrysene), as well as the three PAH studied in our previous Contract No. A732-154 (naphthalene, fluorene and phenanthrene), were photooxidized in a 6500-7000 liter volume all-Teflon chamber (ITC) equipped with two parallel banks (40 lamps per bank) of Sylvania F40/350BL blacklamps for irradiation. The chamber is shown schematically in Figure 7 and the blacklamp spectrum is compared with the solar spectrum in Figure 8, normalized to the same NO_2 photolysis rate. The ITC contains a Teflon-coated fan, rated at 300 liter s^{-1} for rapid mixing of reactants and Pyrex sampling ports for introduction of reactants and collection of gas samples for analyses. Prior to investigation of each of the PAH, the ITC was either cleaned by flushing with purified Riverside ambient air at approximately 400 liter min^{-1} for several days, with the blacklamps being on at their maximum

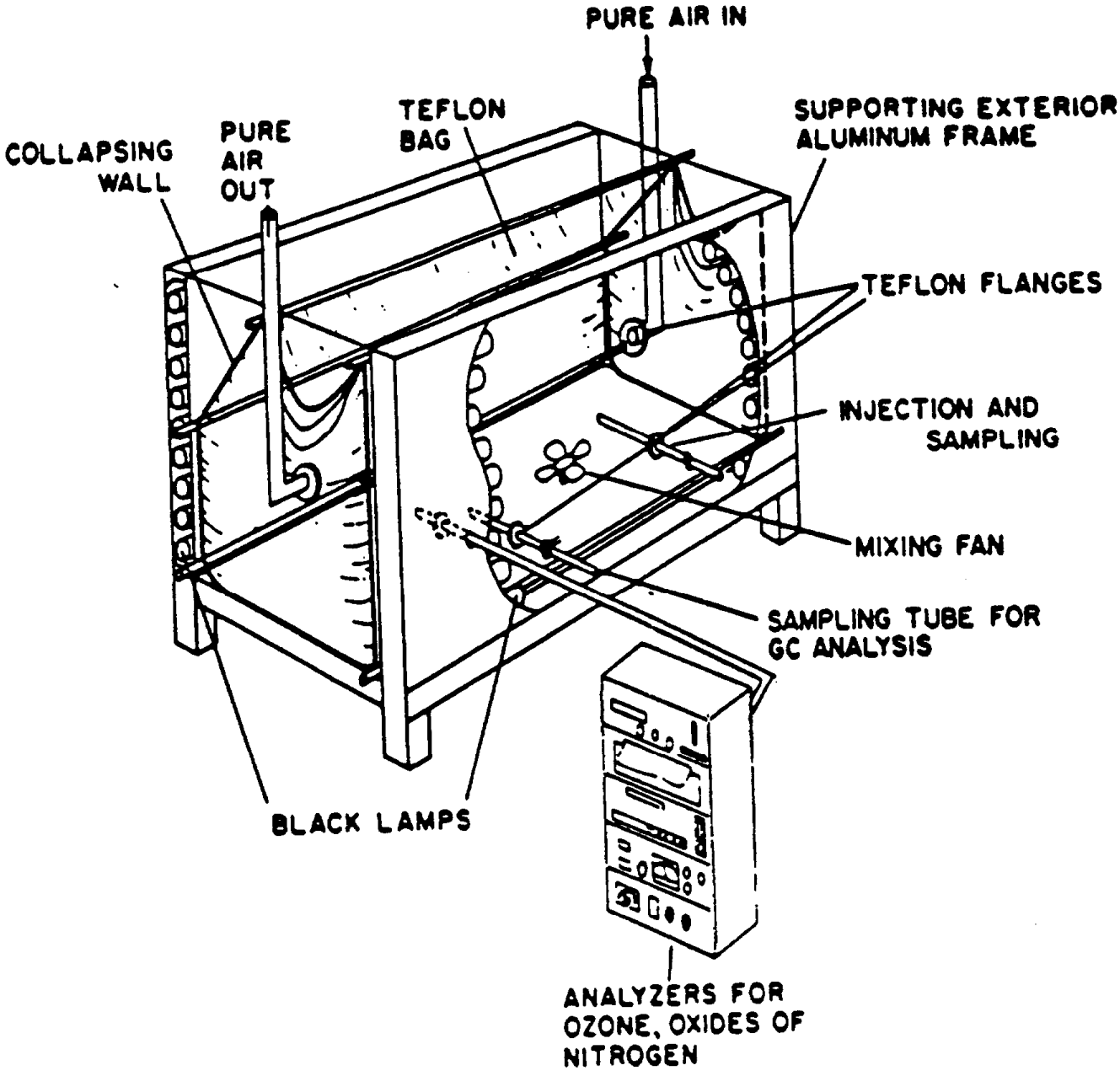


Figure 7. Approximately 6000-liter all-Teflon environmental chamber.

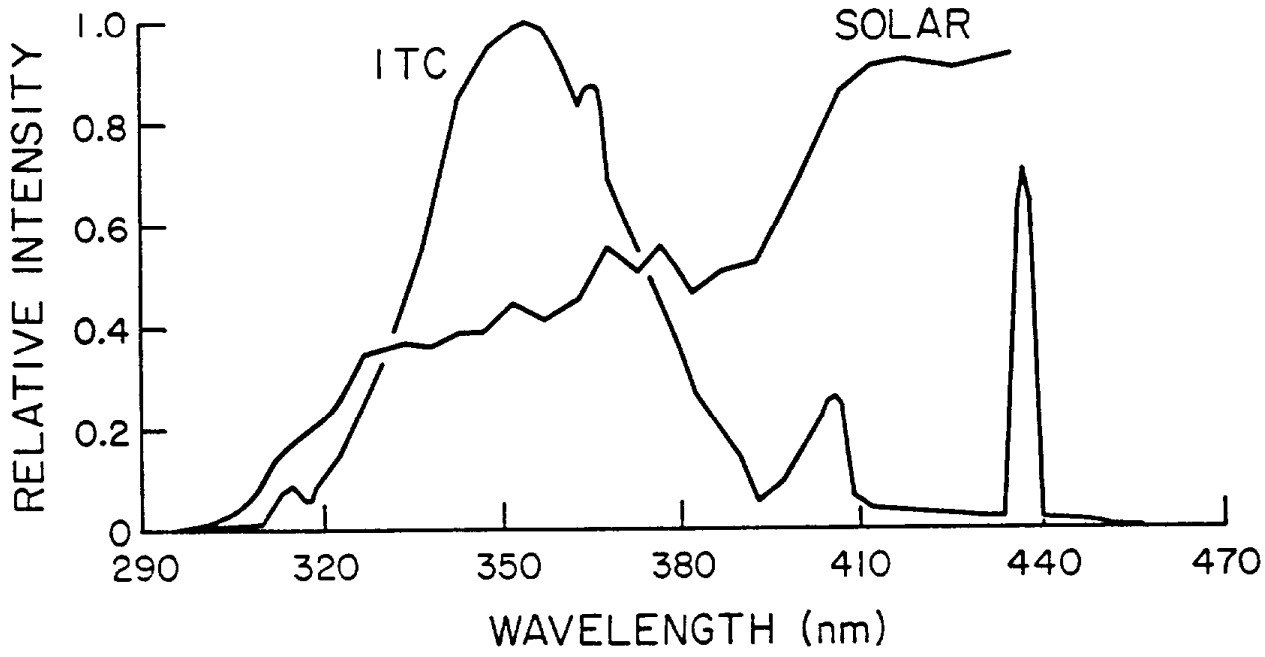


Figure 8. Spectral distribution of the ~6000 ℓ all-Teflon chamber (ITC) blacklamps and the calculated tropospheric solar spectral distribution for a zenith angle of 40° at the solar equinox (SOLAR), both normalized to give the same NO_2 photolysis rate.

intensity for a significant fraction of this flushing time, or a new Teflon chamber was installed and flushed with purified ambient air for several (>20) hrs, again with the blacklamps on for a significant fraction of the pure-air flush. The photooxidations of the PAH were initiated by generating hydroxyl (OH) radicals from the photolysis of methyl nitrite (CH_3ONO) in air at wavelengths >300 nm,

$$CH_3ONO + h\upsilon \rightarrow CH_3O + NO \quad (1)$$

$$CH_3O + O_2 \rightarrow HCHO + HO_2 \quad (2)$$

$$HO_2 + NO \rightarrow OH + NO_2 \quad (3)$$

with this method of generation of OH radicals leading to OH radical concentrations in the chamber of $\sim$(1-2) x 10^8 molecule cm^{-3} [4-8 parts-per trillion (ppt) mixing ratio] (Atkinson *et al.*, 1989), a factor of $\sim$100 higher than in the ambient atmosphere (Prinn *et al.*, 1992). NO was also added to the reactant mixtures, and the initial CH_3ONO and NO mixing ratios in the chamber were 2 ppm and 1 ppm, respectively, and irradiations were carried out for 10 mins (15 mins for fluorene) at the maximum light intensity. Irradiations of CH_3ONO-NO-air mixtures, in the absence of the PAH, were also carried out as "chamber controls".

The use of initial CH_3ONO and NO mixing ratios of 2 ppm and 1 ppm, respectively, and the 10 min irradiation times at maximum light intensity (15 mins for fluorene) were designed to lead to conversion of NO to NO_2 through reaction (3) and the reactions of NO with peroxy radicals generated from the reactions of the PAH. The photolysis of NO_2

$$NO_2 + h\upsilon \rightarrow NO + O(^3P) \quad (4)$$

$$O(^3P) + O_2 + M \rightarrow O_3 + M \qquad (M = air) \quad (5)$$

leads to the formation of ozone and hence the formation of nitrate (NO_3) radicals.

$$O_3 + NO_2 \rightarrow NO_3 + O_2 \qquad (6)$$

Therefore, the photooxidation of the PAH resulted in the PAH being exposed to photolysis and reaction with OH radicals, NO_3 radicals and O_3, as occurs in ambient air (Atkinson, 1988).

The PAH were introduced into the chamber by one of two methods. For the volatile PAH, naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, acenaphthene, acenaphthylene, biphenyl, fluorene and dibenzothiophene, a stream of N_2 gas was passed through a Pyrex tube (0.25 in o.d.) containing the solid PAH for time-periods of ~30 mins to 15 hrs, depending on the volatility of the PAH. For the less volatile PAH, phenanthrene, anthracene, 2-methylphenanthrene, retene, fluoranthene, pyrene, benz(a)anthracene and chrysene, a solution of ~35 mg of the PAH being studied in 100 mL of methanol was sprayed into the chamber, and the majority of the methanol removed by flushing the chamber with purified air for ~30 mins.

The concentrations of the PAH were measured before and after the irradiations by gas chromatography with flame ionization detection (GC-FID), except for benz(a)anthracene and chrysene which were analyzed by combined gas chromatography-mass spectrometry (GC/MS). Gas samples of 100-2090 cm^3 volume were collected from the chamber onto Tenax-TA solid adsorbent, with subsequent thermal desorption and GC analysis, with the larger volume samples being collected for the less volatile PAH. The Tenax samples were thermally desorbed at 250-300 °C onto a 15 m DB-5 megabore column (Hewlett Packard (HP) 5710 or 5880 GC), held at typically 40 °C and temperature programmed at 20 °C min^{-1}. For benz(a)anthracene and chrysene, the samples collected onto the Tenax solid adsorbent were thermally desorbed onto a 60 m DB-5ms fused silica capillary column in a HP 5890 GC interfaced to a HP 5970 Mass Selective Detector (MSD) operated in the scanning mode (45-300 amu). The 60 m DB-5ms column (0.25 mm i.d. and 0.25 μm film thickness) was initially held at 0 °C and temperature programmed at 20 °C min^{-1} to 300 °C. NO and the initial NO concentrations were measured using a chemiluminescence NO-NO_x analyzer.

B. Large Volume Sample Collection, Extraction and HPLC Fractionation

After the CH_3ONO-NO-PAH-air and CH_3ONO-NO-air irradiations, large volume (1000-2500 liters) gas samples were collected from the chamber onto two polyurethane foam (PUF)

plugs in series, using a 7.5 cm diameter x 19 cm long Pyrex tube containing the PUF plugs connected directly to a high-volume sampler motor. With this sampling arrangement, gas samples were collected at ~500-1200 liters min^{-1}. The volumes collected from a given experiment were calculated from the NO_x concentrations measured after the sample had been collected and after the chamber had been back-filled with purified air, using the relationship

$$\text{Volume collected} = 6700\ (1 - [NO_x]_2/[NO_x]_1) \qquad \text{(I)}$$

where the filled chamber volume is taken as 6700 liters (to within ±300 liters), $[NO_x]_1$ is the measured NO_x concentration in the chamber after the sample had been collected and $[NO_x]_2$ is the measured NO_x concentration in the chamber after back-filling to approximately the same volume as prior to sample collection. These PUF plug samples were extracted and fractionated by high-performance liquid chromatography (HPLC) as described by Arey *et al.* (1992) and Harger *et al.* (1992). The PUF plugs were extracted with dichloromethane (CH_2Cl_2) for 4 hrs using a Soxhlet extractor. The CH_2Cl_2 extracts of the PUF plugs were filtered through 0.45 μm Acrodisc Teflon filters, and fractionated by normal-phase HPLC using a semipreparative Regis Spherisorb SSW silica column, 10 cm x 10 mm.

The HPLC instrumentation consisted of a Spectra-Physics Model 8100 gradient liquid chromatograph with a Model 8400 UV/VIS detector (λ = 254 nm) and an ISCO fraction collector. The solvent program (at a flow rate of 3 mL min^{-1}) was: initially 100% hexane for 10 mins, followed by a 5-min gradient to 95% hexane and 5% CH_2Cl_2. The solvent was programmed over the next 25 min to 100% CH_2Cl_2, where it was held for 10 mins, then programmed to 100% acetonitrile over 10 mins, held isocratic for 10 mins, and then programmed back to the initial conditions. Beginning after 1 min, nine 9-min fractions of increasing polarity were collected for bioassay testing.

C. Bioassay Testing

The HPLC fractions were tested for mutagenicity using *Salmonella typhimurium* strain TA98 without exogenous (S9) activation and using the Kado microsuspension preincubation modification (Kado *et al.*, 1983,1986; Arey *et al.*, 1992; Harger *et al.*, 1992) of the histidine-

reversion bioassay of Ames and coworkers (Ames *et al.*, 1975; Maron and Ames, 1983). The HPLC fractions were dissolved in CH_2Cl_2 and solvent-exchanged into dimethylsulfoxide (DMSO), combined with 100 μL ($\sim 1.1 \times 10^{10}$ cells mL^{-1}) aliquots of the resuspended cell culture and incubated for 90 min at 37 °C with vigorous shaking (180 rpm). Following the addition of 2.0 mL of soft agar and mixing, each sample was overlayed onto minimal glucose plates which were incubated at 37 °C for 63 hrs and scored by means of an automatic colony counter. As noted above, for each PAH photooxidation conducted, a chamber control CH_3ONO-NO-air irradiation with the same initial CH_3ONO and NO concentrations was carried out and the HPLC fractions of the PUF plug extracts bioassayed as for the experiments involving the PAH. Generally, for each PAH photooxidation bioassay, a set of HPLC fractions with no added extract (HPLC blank) was also bioassay tested. The PAH fractions, the chamber control fractions and the HPLC blank fractions were all bioassayed on the same day in one mutagenicity test along with the positive control mutagen, 2-nitrofluorene.

In order to reserve the majority of each HPLC fraction for chemical analysis, mutagenicity testing was done on single plates using 4 doses spanning 3 orders of magnitude (e.g., 0.01%, 0.1%, 1.0%, and 10% of the sample per plate). The amount of each HPLC fraction reserved for chemical analysis ranged from 45% to 95% and was chosen beforehand based on the anticipated mutagenicity of known products and the concentration of PAH obtained in the chamber reaction. To obtain net revertants, the average number of spontaneous revertants (from 6 replicates) was subtracted from all counts and the net revertants were used to calculate the dose-response curve. The initial linear portion of the dose-response curve was determined by the criterion that a least-squares analysis yielded an intercept within four standard deviations of the spontaneous background revertants; if not, the highest dose point(s) was eliminated until this criterion was met. The slope from this initial linear portion of the dose-response curve was used to calculate the activity. An as example, the dose-response curves obtained for the pyrene reaction, ITC-2061, is shown in Figure 9.

Toxicity was determined by examining the background lawn of unreverted bacteria on the petrie dish under a dissecting scope and those plates exhibiting a dimished background lawn were not used in the linear regression. This technique will not recognize subtle toxic effects such as those from bacteriostatic compounds which alter the growth kinetics of the test bacteria.

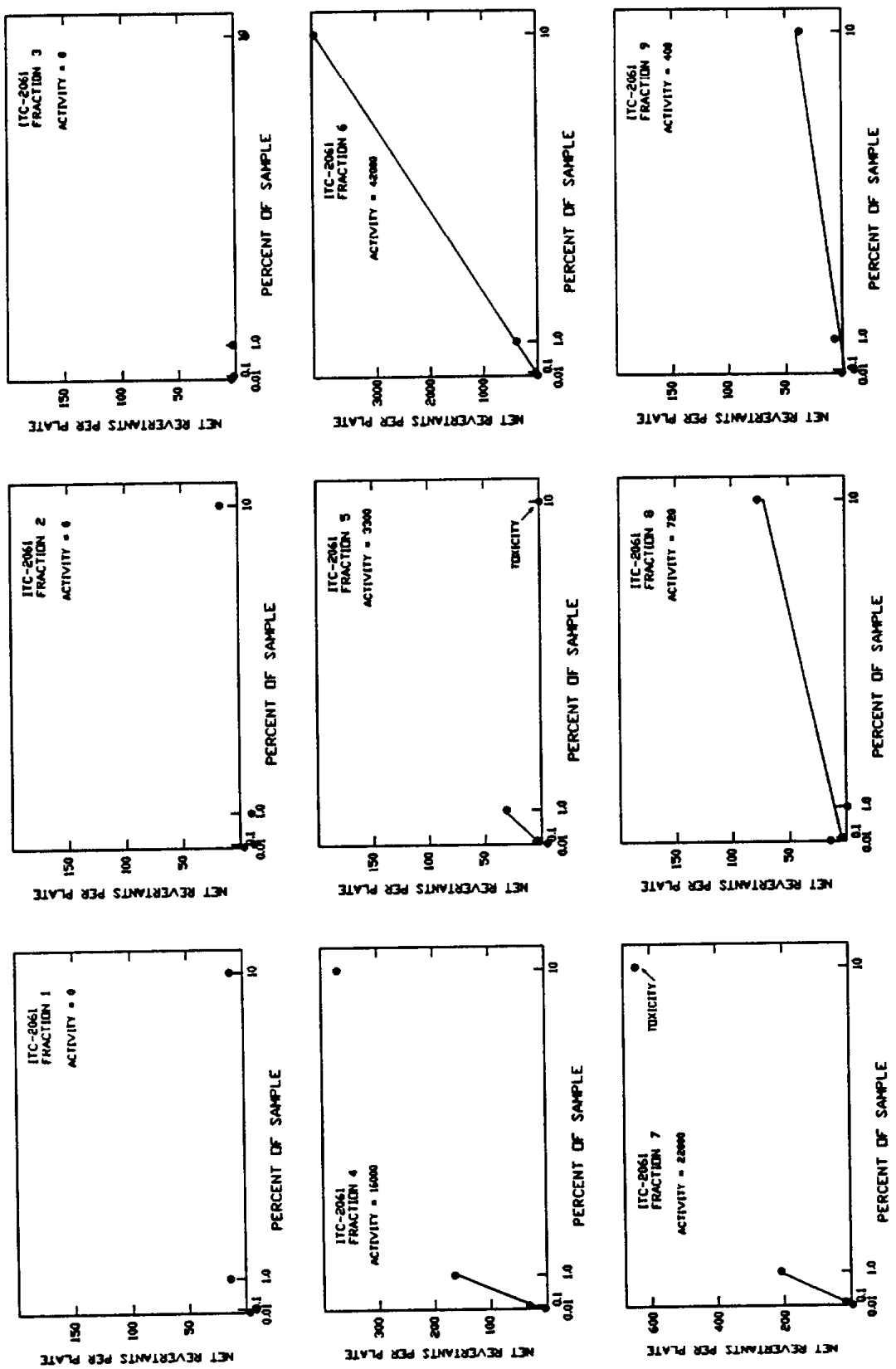


Figure 9. Dose-response curves from the mutagenicity test of HPLC fractions of ITC-2061 (pyrene).

The chamber blanks (the irradiated CH_3-ONO-NO-air mixtures) and the HPLC blanks were tested with one dose per HPLC fraction which was equivalent to the highest of the four doses chosen for the corresponding PAH irradiation. The positive control mutagen, 2-nitrofluorene, was tested with three plates at eight doses (2, 4, 6, 8, 10, 12, 14, and 16 ng $plate^{-1}$).

D. Chemical Analysis

Gas Chromatography/mass spectrometry analysis of selected HPLC fractions, generally including those of highest mutagenic activity, was conducted for several of the PAH photooxidations. The GC/MS analyses were carried out on 60 m DB-5ms fused silica capillary columns (0.25 mm i.d. and 0.25 μm film thickness) using HP 5890 GCs interfaced to either a HP 5970 or HP 5971 MSD. In all cases, the samples were in CH_2Cl_2 solution and all injections were made in the cool on-column mode.

E. Chemicals

The chemicals used, and their stated purities, were: acenaphthene (99%), acenaphthylene (95%), anthracene (99.9+%), biphenyl (99%), chrysene (98%), fluoranthene (98%), 1-methylnaphthalene (99%), 2-methylnaphthalene (98%), 2-methylphenanthrene (99%), pyrene (99%), Aldrich Chemical Company; benz(a)anthracene, Eastman; retene (98%), ICN-KOR; and NO ($\geq$99.0%), Matheson Gas Products. Methyl nitrite was prepared and stored as described by Atkinson *et al.* (1981).

RESULTS

I. PHENANTHRENE: AMBIENT ANALYSES OF POLAR MUTAGENIC REACTION PRODUCTS AND MECHANISTIC STUDIES

A. Analyses of Ambient Air Samples and the SRMs

2-Nitrodibenzopyranone was observed in all of the southern California ambient air filter and PUF plug samples analyzed, and 4-nitrodibenzopyranone was observed in the majority of these samples. Both isomers were observed in the SRM 1649 urban dust. The results of the quantitative analyses of these southern California air samples are listed in Table 1, and the results of analyses of the SRM 1649 urban dust collected in Washington, DC are given in Table 2. 2-Nitrodibenzopyranone was quantified in all of the ambient air samples analyzed, at concentrations ranging between 0.04 and 0.8 ng m^{-3}. In certain samples the GC/MS ion signal for 4-nitrodibenzopyranone was insufficient for peak integration, and hence upper limits equal to the estimated detection limit of ~ 0.02 ng m^{-3} are given.

Our data (Table 1) show that 2-nitrodibenzopyranone was always more abundant than the 4-isomer in the ambient air particulate samples analyzed, generally by a factor of ~ 2. The abundance ratio of 2-nitrodibenzopyranone/4-nitrodibenzopyranone of ~ 2 in these ambient air samples is, however, different than we observed in samples collected from environmental chamber OH radical-initiated reactions of phenanthrene, where the 2-nitrodibenzopyranone/4-nitrodibenzopyranone ratio was ~ 0.5 [Helmig *et al.*, 1992a and Table 3].

The data in Table 1 also show that 2- and 4-nitrodibenzopyranone are distributed between the gas and particle phases (although it should be noted that this is an "operational" definition based on collection on filters (particle phase) and solid adsorbents (gas phase), and may be affected by adsorption of gas-phase nitrodibenzopyranones onto the filters and/or volatilization of the particle-associated nitrodibenzopyranones from the filters during collection). Such a distribution of PAH-derivatives of molecular weight (M.W.) 241 between the gas- and particle-phases at the temperatures typical of southern California is expected based on previous measurements of gas- and particle-phase PAH and nitroarenes in the atmosphere (Arey *et al.*, 1987; 1989a; Atkinson *et al.*, 1988; Bidleman, 1988). Thus, for example, as anticipated, fluoranthene and pyrene (M.W. 202 PAH) are mainly in the gas-phase under southern California conditions, as are the nitrofluorenes (M.W. 211), while benzo[a]pyrene and benzo[e]pyrene

Table 1. Concentrations of 2- and 4-nitrodibenzopyranone (NDBP) in ambient air particulate (filter) and gas-phase (PUF) samples collected in Claremont, CA (1987), Long Beach, CA (1987) and Riverside, CA (1991).

	Sampling Time[a]	Filter		PUF	
		2-NDBP (ng m^{-3})	4-NDBP (ng m^{-3})	2-NDBP (ng m^{-3})	4-NDBP (ng m^{-3})
Claremont					
August 27	D	0.41	0.24	0.14	0.03
August 28	D	0.37	0.21	0.14[b]	0.02[b]
August 29	D	0.24	0.18		
Long Beach					
June 19	D	0.16	0.8		
June 19	N	0.04	<0.02[c]		
June 24	D	0.20	0.07		
June 25	N	0.10	0.04		
July 13	D	0.19	0.09		
July 14	N	0.05	<0.02[c]		
November 11	N	0.12	0.05		
November 13[d]	D	0.13/0.09	0.05/0.03		
December 2	N	0.08	0.06		
December 10	N	0.07	0.05		
Riverside[e]					
September 17	N	0.29	0.19		
September 18	D	0.30	0.20		
September 18	N	0.15	0.08		
September 19	D	0.30	0.14		
September 19/20	N/D[f]	0.25	0.15		
October 14	N	0.36	0.15	0.12	<0.02[c]
October 15	D			0.10	0.03
October 16	N	0.32	0.10	0.03	<0.02[c]
October 17	D	0.26	0.11	0.06	<0.02[c]
October 17	N			0.02	<0.02[c]
October 18	D	0.8[g]	0.21	0.07	<0.02[c]

[a]D = Daytime sample collected from 6 to 18 hr.
N = Nighttime sample collected from 18 to 6 hr.
Date refers to beginning of sampling period.
[b]45% of the sample extract was spiked after HPLC separation.
Sample loss occurring during sample workup (~10-20%) is not corrected for.
[c]Estimated limit of detection.
[d]Co-located replicate filter samples.
[e]Daytime samples from ~8 to 20 hr and nighttime samples from ~20 to 8 hr.
[f]24 hr sample.
[g]Concentration above calibration range.

Table 2. Concentrations of 2- and 4-nitrodibenzopyranone in the National Institute of Standards and Technology Standard Reference Materials 1649 Urban Dust and 1650 Diesel Exhaust Particles.

Compound	Concentration ($\mu g\ g^{-1}$)[a]	
	SRM 1649 Urban Dust	SRM 1650 Diesel Exhaust
2-Nitrodibenzopyranone	0.82 ± 0.15[b]	~0.2
4-Nitrodibenzopyranone	0.52 ± 0.09[b]	<0.1[c]
2-Nitrofluoranthene	0.60[d]	
1-Nitropyrene	0.06[e]	19 ± 2[e]

[a]Microgram per gram of particles.
[b]Single standard deviation, n = 3.
[c]Estimated limit of detection.
[d]Ramdahl *et al.* (1986).
[e]May *et al.* (1992).

(M.W. 252 PAH) and the nitrofluoranthenes and nitropyrenes (M.W. 247) exist essentially totally in the particle phase (Arey *et al.* 1987; Atkinson *et al.*, 1988).

Table 2 shows that the 2- and 4-nitrodibenzopyranones were present in the SRM 1649 urban dust, again with the 2-isomer being more abundant than the 4-isomer. In the SRM 1650 diesel particulate material, 2-nitrodibenzopyranone was quantified at a concentration lower by a factor of ~ 4 than in the SRM 1649 urban dust. The analyses of the diesel SRM were rendered more difficult because of interferences from other organic compounds present, and only an upper limit for the concentration of the 4-nitrodibenzopyranone could be obtained.

B. Environmental Chamber OH Radical-Initiated Experiments

In an initial series of experiments (ITC-1712 through -1721), irradiations of CH_3ONO-NO-phenanthrene-air mixtures were carried out at the maximum light intensity for 10 min. The initial reactant concentrations and the measured final phenanthrene and NO concentrations are given in Table 3 for those experiments for which complete chemical analyses were conducted. For most of these reactions, the majority of the initially present NO was converted to NO_2, thus leading to the formation of O_3 and hence of NO_3 radicals. Thus these experiments simulated atmospheric conditions under which OH radicals, O_3 and NO_3 radicals are all present at significant concentrations. 2- and 4-Nitrodibenzopyranone (Arey *et al.*, 1992; Helmig *et al.*, 1992a), 6H-dibenzopyran-6-one and 2,2´-diformylbiphenyl were observed as products and quantified from these reactions (Table 3). The estimated amounts of phenanthrene reacted (see footnotes to Table 3) and the product formation yields (which do not take into account any photolysis and/or reactive losses of the products) are also given in Table 3.

A second series of experiments (ITC-1889 through -1894) were carried out using conditions designed to avoid the formation of O_3 and NO_3 radicals. In these experiments, significantly lower amounts of phenanthrene were estimated to have reacted (Table 3) and only upper limits for the 2- and 4-nitrodibenzopyranone concentrations and formation yields could be obtained (Table 3). The data from these two series of experiments, including the possibly lower formation yield of 4-nitrodibenzopyranone in the second series compared to the first series, may be consistent with the nitrodibenzopyranones not being formed as "first-generation" products from the OH radical-initiated reaction of phenanthrene. These data, however, do not allow the

Table 3. Experimental conditions and nitrodibenzopyranone (NDBP), dibenzopyranone (DBP), and 2,2´-diformylbiphenyl (DFBP) formation from the environmental chamber OH radical-initiated reactions of phenanthrene, DBP and DFBP (taken from Helmig *et al.*, 1992b).

	10^{13} x Initial Conc. (molecule cm^{-3})				10^{13} x Final Conc. (molecule cm^{-3})			Product Yield %			
Compound ITC Run #	CH_3ONO	NO	Reac-tant	Irrad. time min.	NO	Reac-tant	Compound reacted[a] (molecule)	2-NDBP	4-NDBP	DBP	DFBP
Phenanthrene											
1714	4.8	3.2	0.39	10		0.074	1.1×10^{19}				
1715	5.6	2.3	0.31	10	0.48	0.19	1.9×10^{19}	0.02%[b]	0.05%[b]		
1716	5.0	2.4	0.18	10		0.18	1.2×10^{19}	0.01%	0.04%		
1717	5.4	2.3	0.39	10	1.4	0.39	2.9×10^{19}	0.004%[c]	0.007%[c]	0.1%[d]	0.04%[d]
1718	5.0	2.9	0.36	10	0.36	0.41	2.0×10^{19}	0.01%[c]	0.02%[c]	0.2%[d]	0.1%[d]
1719	5.2	2.4	0.41	10	0.35	0.34	2.6×10^{19}	0.008%[c]	0.01%[c]	0.3%[d]	0.1%[d]
1889	24.9	20.8	0.28	1	2.4	0.27	1.2×10^{18}	<0.03%	<0.03%	<0.03%[d]	
1890	23.6	24.0	0.10	2	11.3	0.05	5.5×10^{17}	<0.06%	<0.06%	0.2%[d]	
1891	24.8	23.2	0.17	5	0.02	0.07	2.4×10^{18}	<0.02%	<0.02%		
Dibenzopyranone											
1842	5.6	2.9		10	≤0.02	0.06[e]	8.1×10^{18}	0.03%[c]	≤0.007%[c]		
2,2´-Diformylbiphenyl											
1964	5.1	2.0		10		0.014[e]	1.3×10^{18}	0.1%[c]	~0.02%[c]	1%[d]	

(continued)

[a] Since the phenanthrene, dibenzopyranone and 2,2´-diformylbiphenyl reactants were sprayed onto the chamber walls prior to the experiments, the amounts of compound reacted cannot be obtained solely from the measured gas-phase concentrations (note that for phenanthrene, in many cases the initial and final measured gas-phase concentrations are similar). The amounts of compound reacted were calculated from the expression:

$$\text{Compound Reacted} = 0.5([\text{reactant}]_{\text{initial}} + [\text{reactant}]_{\text{final}}) \times k^{OH}[OH](t - t_o)V$$

where V is the volume sampled (1700 liters for ITC-1714 through -1719, 2000 liters for ITC-1889 through -1891, 3860 liters for ITC-1842 and 2000 liters for ITC-1964), k^{OH} is the rate constant for reaction of the OH radical with the reactant, and [OH] is the average OH radical concentration during the irradiation. Rate constants k^{OH} (in units of 10^{-11} cm^3 $molecule^{-1}$ s^{-1}) of 3.1 (experimentally measured) for phenanthrene (*37*) and 3 (estimated) for dibenzopyranone and 2,2´-diformylbiphenyl (*38*) were used. The OH radical concentration was taken to be [OH] = 1.0×10^8 ($[CH_3ONO]_{initial}/[NO]_{initial}$) molecule cm^{-3}, based on the data obtained from CH_3ONO-NO-NO_2-air irradiations of benzene and toluene conducted at the maximum light intensity for irradiation times of 5-15 min (*39*). For runs ITC-1889 through -1894, $\sim 1.2 \times 10^{13}$ molecule cm^{-3} of cyclohexane was included in the reactant mixtures to monitor the OH radical concentrations. For experiments, the OH radical concentrations calculated from [OH] = 1.0×10^8 ($[CH_3ONO]_{initial}/-[NO]_{initial}$) molecule cm^{-3} are within a factor of 2 (being a factor of 1.8 ± 0.5 (single standard deviation higher) of those derived from the measured initial and final cyclohexane concentrations. The given amount of compound reacted may therefore be lower by up to a factor of 2 and the cited product formation yields may be higher by up to a factor of 2.

[b] From a composite of PUF plug extracts of runs ITC-1714 and -1715.

[c] Samples spiked with an internal standard after extraction and HPLC fractionation. Cited concentrations are anticipated to be low by ~20-30%.

[d] Quantified by GC-FID with an external standard calibration on the final extract of the HPLC fractionation. Cited concentrations are anticipated to be low by ~20-30%.

[e] Determined from analyses of the PUF plug samples. This concentration was used to calculate the amount of compound reacted, assuming that $[\text{reactant}]_{\text{initial}} = [\text{reactant}]_{\text{final}}$.

importance of photolysis of the nitrodibenzopyranones for the spectral distribution of our chamber light source (Arey *et al.*, 1990; Atkinson *et al.*, 1990a) to be assessed.

Dibenzopyranone and 2,2´-diformylbiphenyl were observed as products in runs ITC-1717 through -1719, with approximate formation yields of ~0.2% and ~0.1%, respectively. Since these compounds could serve as possible precursors to the nitrodibenzopyranones, OH radical reactions of these compounds were carried out in the presence of NO_x, using initial CH_3ONO and NO concentrations and irradiation conditions similar to those used in the first set of phenanthrene experiments (ITC-1712 to -1721). The relevant experimental data are given in Table 3. The estimated formation yields of the 2- and 4-nitrodibenzopyranones from both the dibenzopyranone and the 2,2´-diformylbiphenyl were <1% (Table 3). Due to the low formation yields of the dibenzopyranone and the 2,2´-diformylbiphenyl as primary products, the nitrodibenzopyranone formation yields from these compounds are obviously too low for secondary formation to account for the entire amounts observed in the phenanthrene experiments ITC-1714 through -1719. Furthermore, the OH radical-initiated reactions of both dibenzopyranone and 2,2´-diformylbiphenyl led to 2-nitrodibenzopyranone/4-nitrodibenzopyranone ratios of ≥5, much higher than observed from the phenanthrene reaction.

C. Contribution of Nitrodibenzopyranones to Ambient Mutagenicity

The ambient air concentrations of 2- and 4-nitrodibenzopyranone reported here and given in Table 1 can be utilized with their mutagenic activities in the microsuspension modification of the *Salmonella* bioassay of 240,000 revertants μg^{-1} and 2,000 revertants μg^{-1}, respectively (Arey *et al.*, 1992; Harger *et al.*, 1992; Helmig *et al.*, 1992a), to assess the contributions of these two atmospherically-formed phenanthrene derivatives to ambient direct-acting mutagenicity. For reference, the direct-acting activities of 2-nitrofluorene and 1-nitropyrene in the microsuspension assay have been reported to be 19,400 ± 950 (Arey *et al.*, 1992) and 47,300 ± 3,200 (Agurell and Stensman, 1992) revertants μg^{-1}, respectively.

Table 4 gives the mutagenic activities (revertants m^{-3}) of HPLC fraction #6 in which the nitrodibenzopyranones elute for ambient samples collected in Claremont, CA for which bioassays were conducted, together with the calculated revertants m^{-3} due to 2-nitrodibenzopyranone. The contribution of the 4-nitrodibenzopyranone to ambient air mutagenic activity in these samples

Table 4. Calculated contributions of 2-nitrodibenzopyranone (2-NDBP) to the mutagenic activities[a] of the HPLC fraction #6 of ambient air samples.

Sample	Mutagenic Activity (revertants m^{-3})	
	HPLC Fraction 6[b]	2-NDBP
Claremont Filter[c] (8/27/87, Daytime)	73 (47%)	98
Claremont Filter[c] (8/27/87, Daytime)	72 (45%)	89
Claremont Filter[c] (8/29/87, Daytime)	50 (49%)	58
Claremont PUF Plug[d] (8/28/87, Daytime)	41 (20%)	34

[a]Activities measured with the microsuspension modification of the Ames *Salmonella* assay, strain TA98, without microsomal activation.
[b]The values given in parentheses are the percentage contribution of HPLC fraction #6 to the sum of all HPLC fractions.
[c]From Arey *et al.* (1992).
[d]From Harger *et al.* (1992).

was negligible (<1 revertant m^{-3}). It can be seen from Table 4 that 2-nitrodibenzopyranone accounts for all of the fraction #6 activity for the particle-phase filter samples and most of the fraction #6 mutagenicity for the gas-phase PUF plug sample. It must be remembered, however, that when dealing with complex mixtures such as the HPLC fractions from ambient extracts, the % contribution may be misleading if the activity is not strictly additive. For example, non-obvious toxic effects (i.e., not sufficient to cause non-linearity in the dose-response curve, but decreasing the overall activity) could cause a decrease in the observed activity of the mixture, enhancing the calculated % contribution from a single compound.

Fraction #6 exhibits the highest mutagenicity of any of the HPLC fractions of the extracts of the filter samples (Arey *et al.*, 1992; Harger *et al.*, 1992; Helmig *et al.*, 1992a). For example, fraction #6 for the Claremont particle-phase filter samples given in Table 4 has $\sim 45\%$ of the total activity (i.e., the sum of the activity of all HPLC fractions) while fraction #4 (the nitroarene-containing fraction) has $\sim 6\%$ of the total activity. In contrast, the HPLC fraction #6 of the vapor phase PUF plug sample has 20% of the total PUF plug activity, while the nitroarene-containing fraction #4 has 50% (Harger *et al.*, 1992). Thus, 2-nitrodibenzopyranone is a significant contributor of ambient air particle- and gas-phase mutagenicity in the microsuspension assay and contributes more than the nitroarenes to the particle-phase direct-acting activity. It is recognized that this large percentage contribution from a single compound may be unique to the assay system used. However, since additional nitro-PAH lactones have been tentatively identified in polar mutagenic subfractions of samples collected in Riverside, CA (Atkinson *et al.*, 1991) and two of the nitropyrene lactone isomers have been found to be over an order of magnitude more mutagenic than 1-nitropyrene in the standard plate-incorporation assay (El-Bayoumy and Hecht, 1986) it is likely that the nitro-PAH lactones will be an important class of ambient mutagens whose health effects and atmospheric formation processes warrant further study.

II. SELECTED PAH: MUTAGRAMS AND CHEMICAL ANALYSES

A series of irradiations of CH_3ONO-NO-PAH-air and CH_3ONO-NO-air mixtures were carried out, with the initial CH_3ONO and NO concentrations being the same in the CH_3ONO-NO-air and CH_3ONO-NO-PAH-air mixtures. For each PAH, two (or more) CH_3ONO-NO-air

irradiations were carried out, with the last of these being chosen as the "chamber blank". Three to six CH_3ONO-NO-PAH-air irradiations were then carried out, with one of these being chosen for bioassay testing, generally on the basis of the GC chamber concentration data and on the perceived quality of the experiment. The measured PAH concentrations (in ppb) before and after the irradiations for the CH_3ONO-NO-PAH-air irradiations chosen for bioassay, and the volumes collected onto PUF plugs for extraction and HPLC fractionation are given in Table 5.

A. Bioassay Results

The mutagenic activities of the HPLC fractions of the CH_3ONO-NO-PAH-air irradiations, the CH_3ONO-NO-air irradiations (chamber blank) and the HPLC controls (HPLC blank) for each chamber photooxidation listed in Table 5 are given in Tables 6-23. For completeness, in addition to the PAH studied under this contract, the data obtained previously (Arey *et al.*, 1992) for naphthalene, fluorene and phenanthrene are also included. Plots (mutagrams) of the percentage of the total mutagenicity per HPLC fraction against the HPLC fraction number (polarity increasing with HPLC fraction number) are shown in Figures 10-27. Two irradiations of 2-methylphenanthrene-CH_3ONO-NO-air were carried out with bioassay testing, and both sets of data are given in the Tables and Figures. For pyrene, two sets of experiments were carried out (ITC-2061 in the first set and ITC-2127 through ITC-2131 in the second set), with no GC-FID analyses of pyrene being carried out for ITC-2061. The mutagrams from the single pyrene experiment (ITC-2061) shown in Figure 24 and the composited experiments (ITC-2127 through ITC-2131) shown in Figure 25 are remarkably similar, showing that the experimental methods used are capable of good reproducibility. The mutagenicity profiles from the replicate 2-methyl-phenanthrene experiments (Figures 19 and 20) are qualitatively similar both showing mutagens in fractions #3 and #4 and more polar mutagens in fractions #6 and #7.

Table 5. Experimental conditions for the irradiation of CH_3ONO-NO-PAH-air mixtures.

PAH	ITC Run #	Gas-phase Concentration (ppb)		Bioassay Sample Volume (liter)
		Pre-Irradiation	Post-Irradiation	
Naphthalene[a]	1698	910	130	1900
1-Methylnaphthalene	2241	1030	80	2000
2-Methylnaphthalene	2216	2150	560	2030
Acenaphthene	2248	474	8	2230
Acenaphthylene	2252	727	34	1620
Biphenyl	2256	734	392	2060
Fluorene[a]	1643	72	11	2300
Dibenzothiophene	2054	9.6	4.3	~2000[b]
Phenanthrene[a]	1649	160		~2000[b]
2-Methylphenanthrene	2172 2173	189 245	95 26	1830 1880
Anthracene	2265	12	5	2550
Retene	2260	21	6	2060
Fluoranthene	2118	~2[c]	~3[c]	1530
Pyrene	2061 2127-2131[e]	d 5.3	d 3.1	~1000 1600
Benz(a)anthracene	2271	~15	15	1650
Chrysene	2274-2276[e]	0.13	0.03	1750

[a]From Contract No. A732-154.
[b]Not measured; estimated to be ~2000 liters.
[c]Estimated from subsequent four irradiations.
[d]No GC-FID analyses performed.
[e]Composites.

Table 6. Mutagenic activities of the products of the gas-phase reaction of naphthalene with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.

HPLC Fraction #	HPLC Column Blank	Chamber Blank ITC-1696	Naphthalene ITC-1698
1	53	49	0
2	40	0	0
3	29	0	0
4	29	67	72,000
5	20	42	5,200
6	184	80	730
7	111	207	11,000
8	113	80	6,200
9	80	2	1,500
SUM	659	527	96,630

Table 7. Mutagenic activities of the products of the gas-phase reaction of 1-methylnaphthalene with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.

HPLC Fraction #	HPLC Column Blank	Chamber Blank ITC-2239	1-Methylnaphthalene ITC-2241
1	49	0	0
2	76	0	0
3	0	0	660
4	85	346	240,000
5	36	0	56,000
6	22	0	3,500
7	499	378	21,000
8	121	18	2,500
9	103	9	320
SUM	991	751	323,980

Table 8. Mutagenic activities of the products of the gas-phase reaction of 2-methylnaphthalene with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.

HPLC Fraction #	HPLC Column Blank	Chamber Blank ITC-2214	2-Methylnaphthalene ITC-2216
1	166	162	0
2	76	67	0
3	72	139	18,000
4	243	409	170,000
5	85	99	0
6	117	175	0
7	175	274	0
8	400	234	550
9	207	256	210
SUM	1,541	1,815	188,760

Table 9. Mutagenic activities of the products of the gas-phase reaction of acenaphthene with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.

HPLC Fraction #	HPLC Column Blank	Chamber Blank ITC-2246	Acenaphthene ITC-2248
1	0	0	0
2	0	99	0
3	0	0	0
4	0	1,152	100,000
5	22	117	3,900
6	9	81	16,000
7	117	324	41,000
8	117	162	550
9	0	157	270
SUM	153	2,092	161,720

Table 10. Mutagenic activities of the products of the gas-phase reaction of acenaphthylene with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.

HPLC Fraction #	HPLC Column Blank	Chamber Blank ITC-2250	Acenaphthylene ITC-2252
1	31	13	0
2	0	36	0
3	0	261	0
4	0	1,422	30,000
5	0	288	1,800
6	0	787	190,000
7	189	846	180,000
8	67	225	3,000
9	45	148	580
SUM	332	4,026	405,380

Table 11. Mutagenic activities of the products of the gas-phase reaction of biphenyl with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.

HPLC Fraction #	HPLC Column Blank	Chamber Blank ITC-2254	Biphenyl ITC-2256
1	9	16	0
2	40	0	0
3	0	29	13,000
4	0	3,174	850
5	45	92	0
6	49	290	760
7	709	247	300
8	353	220	150
9	216	101	72
SUM	1,418	4,169	15,132

Table 12. Mutagenic activities of the products of the gas-phase reaction of fluorene with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.

HPLC Fraction #	HPLC Column Blank	Chamber Blank ITC-1639	Fluorene ITC-1643
1	67	110	0
2	42	0	1,200
3	27	0	130
4	260	110	92,520
5	98	95	15,610
6	51	130	11,000
7	502	220	16,000
8	340	330	250
9	527	250	340
SUM	1,914	1,245	137,050

Table 13. Mutagenic activities of the products of the gas-phase reaction of dibenzothiophene with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.

HPLC Fraction #	HPLC Column Blank	Chamber Blank ITC-2053	Dibenzothiophene ITC-2054
1	16	32	0
2	14	8	0
3	8	0	0
4	0	0	150,000
5	24	4	370
6	26	148	1,000
7	0	0	7,400
8	24	52	97
9	56	0	0
SUM	168	244	158,867

Table 14. Mutagenic activities of the products of the gas-phase reaction of phenanthrene with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.

HPLC Fraction #	HPLC Column Blank	Chamber Blank ITC-1648	Phenanthrene ITC-1699
1	14	0	0
2	4	0	44,000
3	2	0	29,000
4	0	310	21,900
5	17	280	3,420
6	24	340	230,000
7	181	0	12,000
8	43	100	240
9	14	110	180
SUM	299	1,140	340,740

Table 15. Mutagenic activities of the products of the gas-phase reaction of 2-methylphenanthrene with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.*

HPLC Fraction #	Chamber Blank ITC-2170	2-Methylphenanthrene ITC-2172
1	0	95
2	16	0
3	0	8,100
4	33	2,500
5	0	840
6	0	4,400
7	0	1,500
8	169	230
9	156	230
SUM	374	17,895

*No HPLC blank collected.

Table 16. Mutagenic activities of the products of the gas-phase reaction of 2-methylphenanthrene with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.*

HPLC Fraction #	Chamber Blank ITC-2170	2-Methylphenanthrene ITC-2173
1	0	97
2	16	0
3	0	2,000
4	33	1,400
5	0	730
6	0	1,700
7	0	2,900
8	169	570
9	156	370
SUM	374	9,767

*No HPLC blank collected.

Table 17. Mutagenic activities of the products of the gas-phase reaction of anthracene with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.

HPLC Fraction #	HPLC Column Blank	Chamber Blank ITC-2263	Anthracene ITC-2265
1	22	0	0
2	0	9	0
3	0	4	74
4	0	693	1,800
5	72	58	0
6	0	270	180
7	229	112	160
8	175	85	200
9	76	9	86
SUM	574	1,240	2,500

Table 18. Mutagenic activities of the products of the gas-phase reaction of retene with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.

HPLC Fraction #	HPLC Column Blank	Chamber Blank ITC-2258	Retene ITC-2260
1	40	0	170
2	4	0	0
3	40	0	880
4	0	0	540
5	4	49	920
6	22	94	1,900
7	153	49	2,100
8	135	157	340
9	72	49	200
SUM	470	398	7,050

Table 19. Mutagenic activities of the products of the gas-phase reaction of fluoranthene with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.

HPLC Fraction #	HPLC Column Blank	Chamber Blank ITC-2118	Fluoranthene ITC-2119
1	0	0	0
2	0	40	0
3	40	0	0
4	460	400	96,000
5	0	500	16,000
6	60	0	390
7	240	280	2,000
8	0	440	0
9	140	280	0
SUM	940	1,940	114,390

Table 20. Mutagenic activities of the products of the gas-phase reaction of pyrene with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.

HPLC Fraction #	HPLC Column Blank	Chamber Blank ITC-2060	Pyrene + OH ITC-2061
1	20	30	0
2	30	270	0
3	0	0	0
4	120	180	16,000
5	20	200	3,300
6	70	30	42,000
7	20	110	22,000
8	70	130	720
9	330	140	400
SUM	680	1090	84,420

Table 21. Mutagenic activities of the products of the gas-phase reaction of pyrene with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.*[a]

HPLC Fraction #	Chamber Blank ITC-2126	Pyrene + OH ITC-2127-ITC-2131[b]
1	12	0
2	12	0
3	93	1,780
4	0	26,000
5	0	6,400
6	47	78,000
7	142	42,000
8	97	1,240
9	1	260
SUM	404	155,680

[a]No HPLC blank collected.
[b]Five chamber reactions were combined for this test. The tabulated numbers of revertants have been normalized to one chamber reaction for comparison purposes.

Table 22. Mutagenic activities of the products of the gas-phase reaction of benz(a)anthracene with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.

HPLC Fraction #	HPLC Column Blank	Chamber Blank ITC-2268	Benz(a)anthracene ITC-2271
1	36	0	0
2	0	0	0
3	0	0	0
4	4	472	300
5	36	211	110
6	36	234	150
7	247	108	300
8	148	130	260
9	139	108	0
SUM	646	1,263	1,120

Table 23. Mutagenic activities of the products of the gas-phase reaction of chrysene with the OH radical in the presence of NO_x. Tested on TA98 without S9 in the micro-suspension test.

HPLC Fraction #	HPLC Column Blank	Chamber Blank ITC-2273	Chrysene ITC-2274 ITC-2276*
1	0	4	0
2	0	0	0
3	36	27	0
4	0	396	1467
5	36	40	120
6	0	9	160
7	418	54	130
8	558	135	93
9	252	315	83
SUM	1,300	980	2,053

*Three chamber reactions were combined for this test. The tabulated numbers of revertants have been normalized to one chamber reaction for comparison purposes.

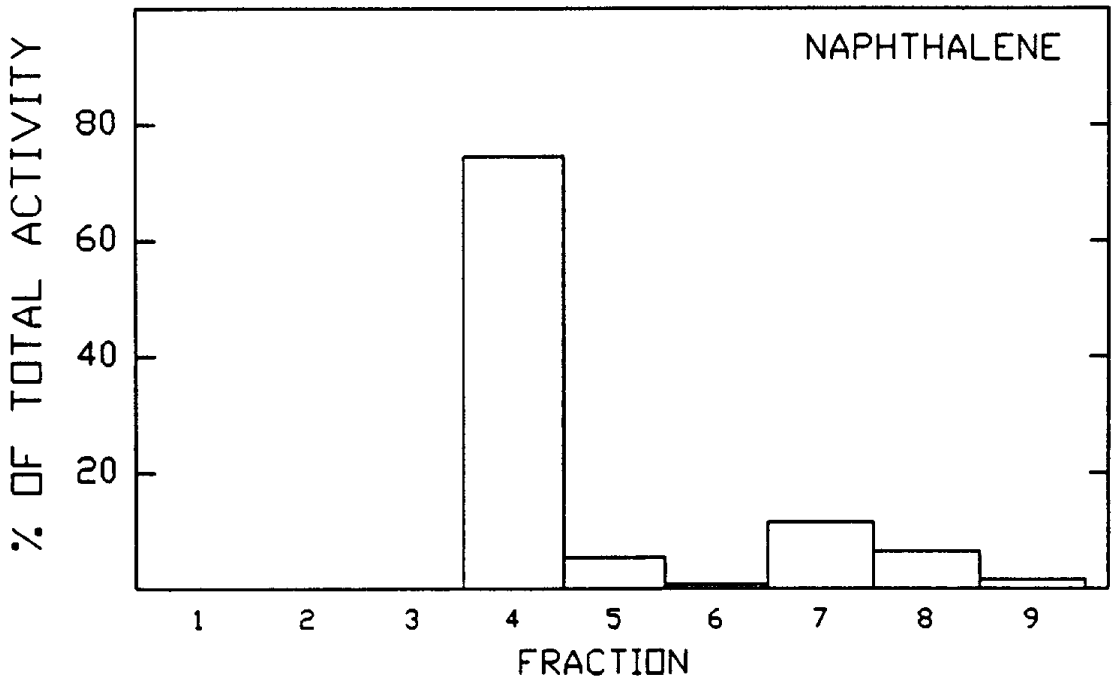


Figure 10. HPLC mutagram from the simulated atmospheric reaction of naphthalene (ITC 1698). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the naphthalene chamber reaction was 96,630 revertants.

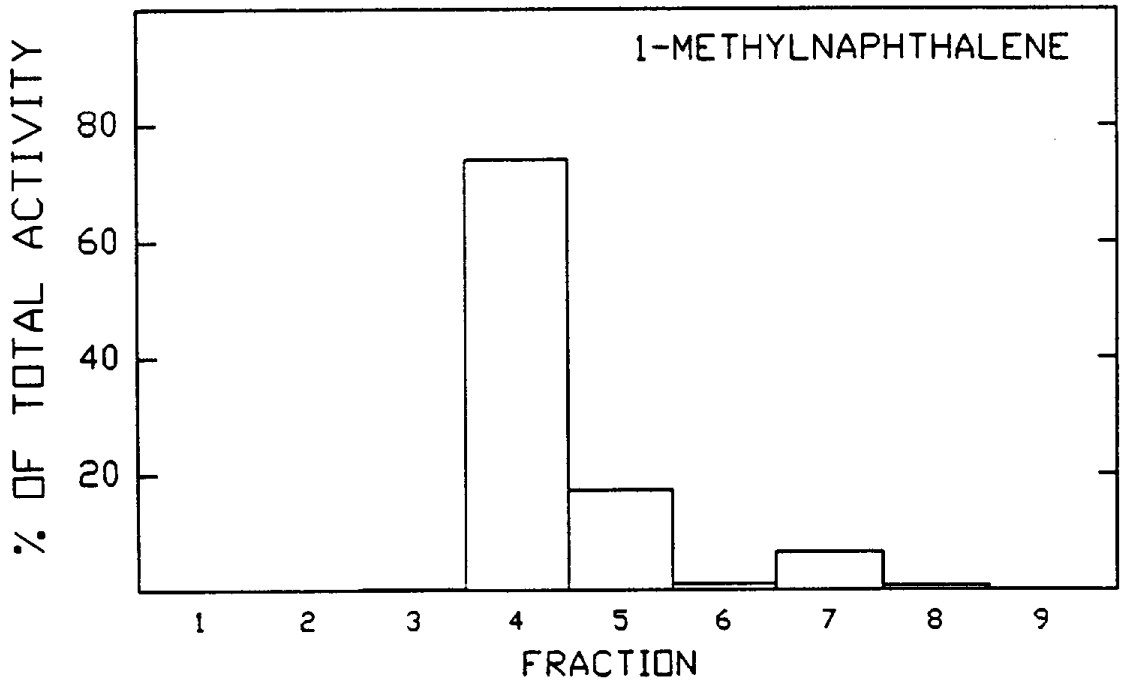


Figure 11. HPLC mutagram from the simulated atmospheric reaction of 1-methylnaphthalene (ITC 2241). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the 1-methylnaphthalene chamber reaction was 323,980 revertants.

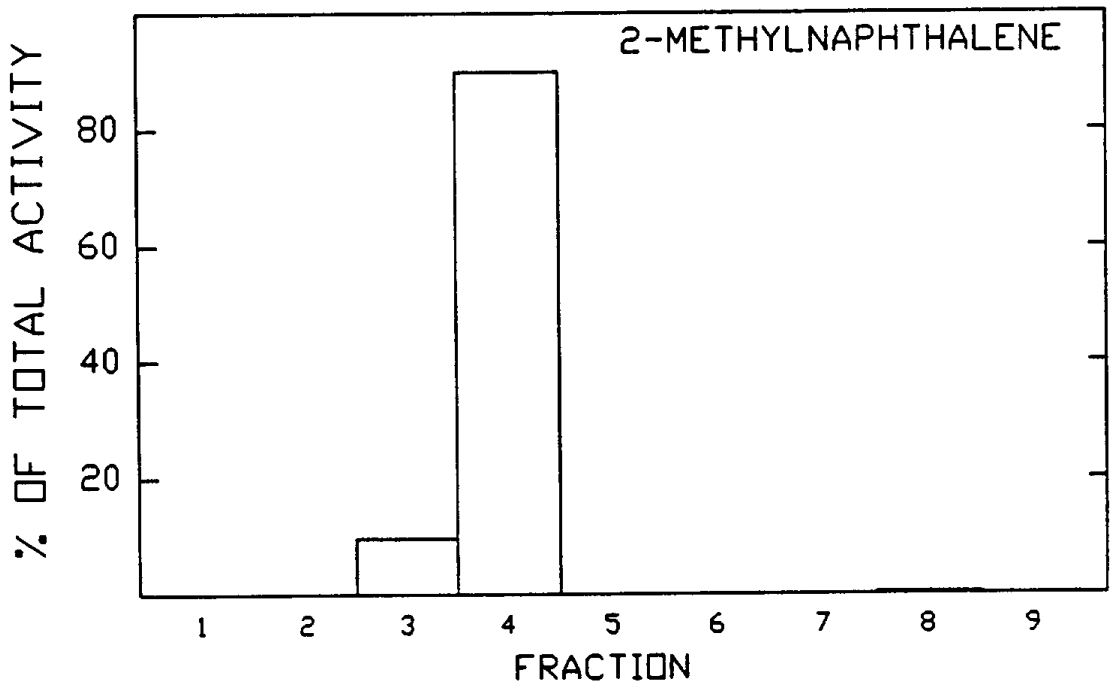


Figure 12. HPLC mutagram from the simulated atmospheric reaction of 2-methylnaphthalene (ITC 2216). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the 2-methylnaphthalene chamber reaction was 188,760 revertants.

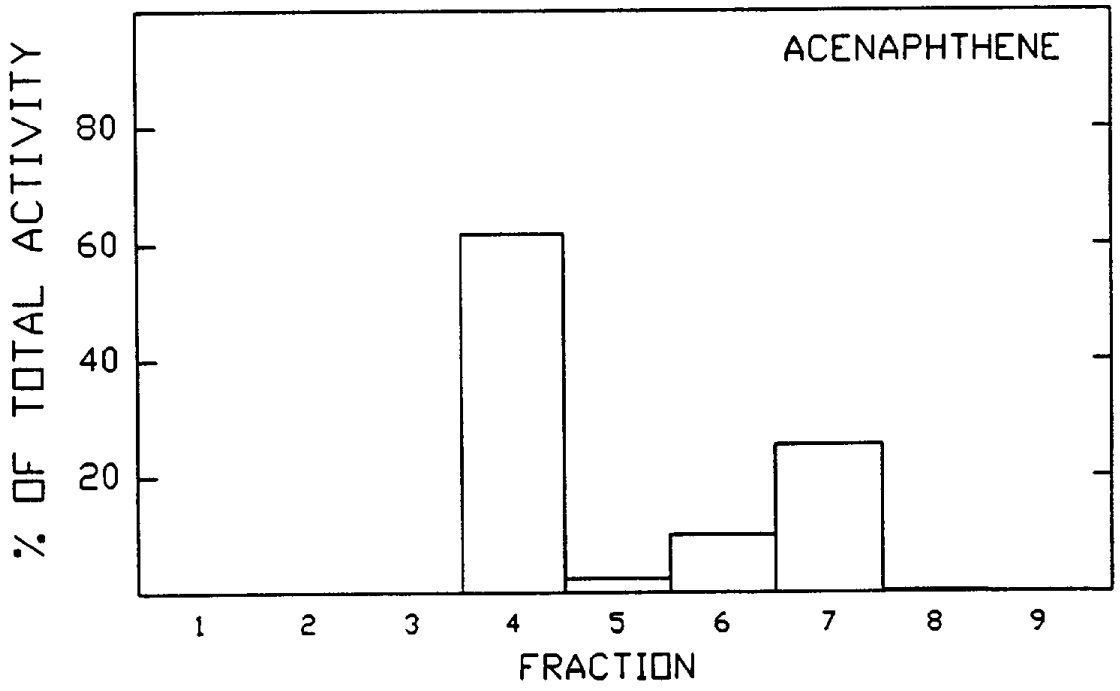


Figure 13. HPLC mutagram from the simulated atmospheric reaction of acenaphthene (ITC 2248). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the acenaphthene chamber reaction was 161,720 revertants.

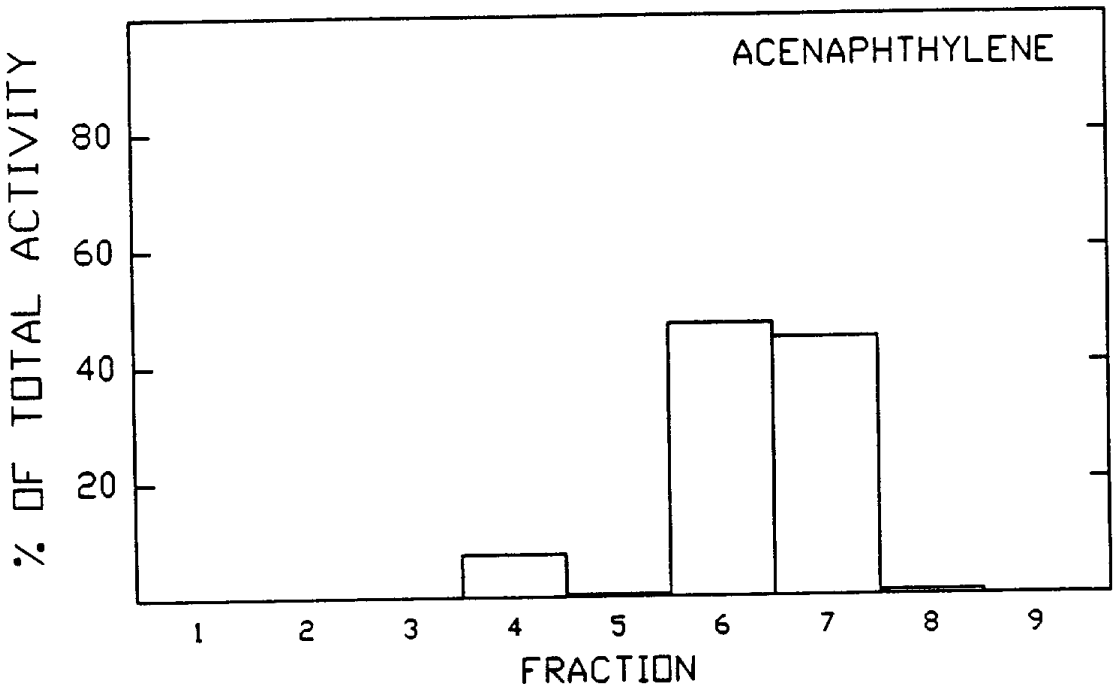


Figure 14. HPLC mutagram from the simulated atmospheric reaction of acenaphthylene (ITC 2252). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the acenaphthylene chamber reaction was 405,380 revertants.

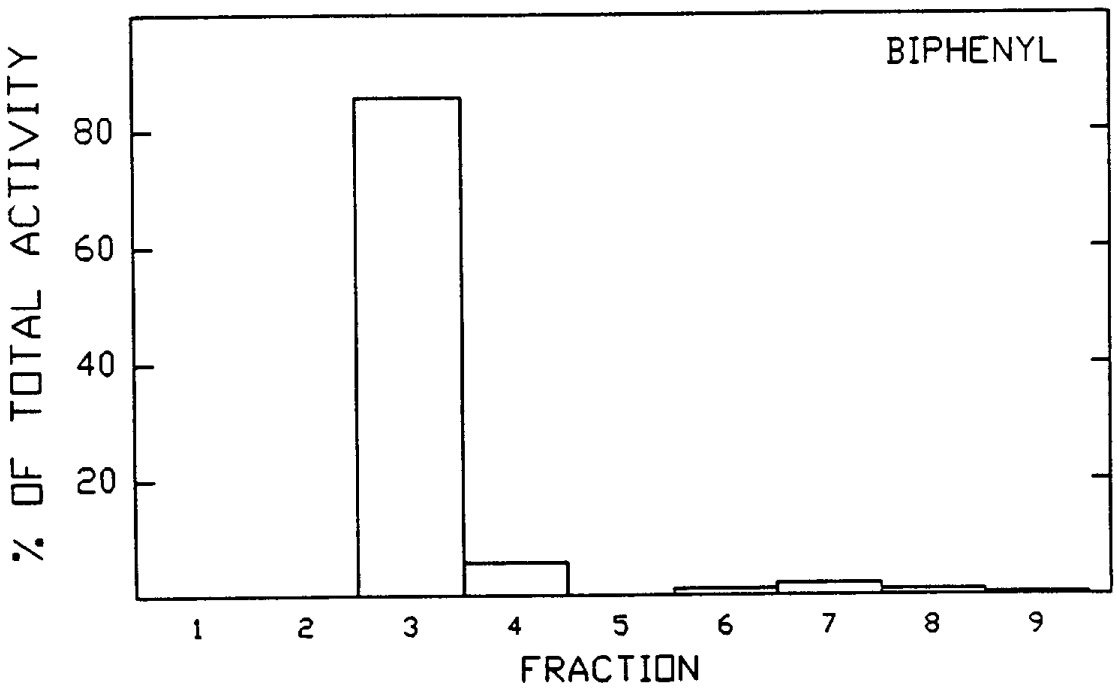


Figure 15. HPLC mutagram from the simulated atmospheric reaction of biphenyl (ITC 2256). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the biphenyl chamber reaction was 15,132 revertants.

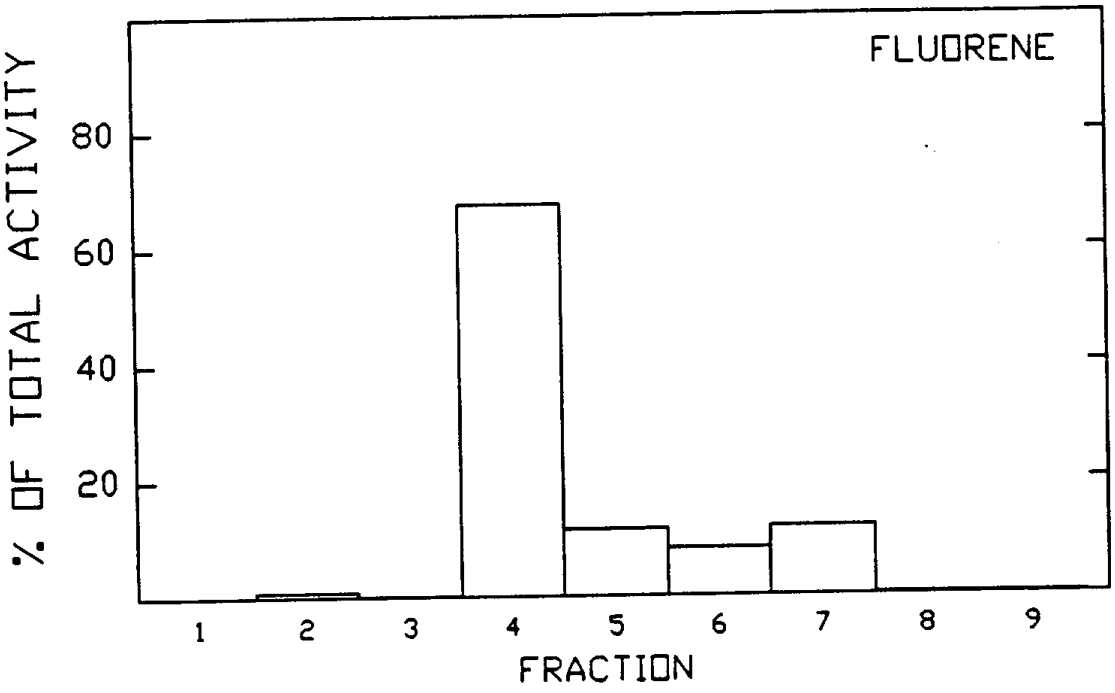


Figure 16. HPLC mutagram from the simulated atmospheric reaction of fluorene (ITC 1643). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the fluorene chamber reaction was 137,050 revertants.

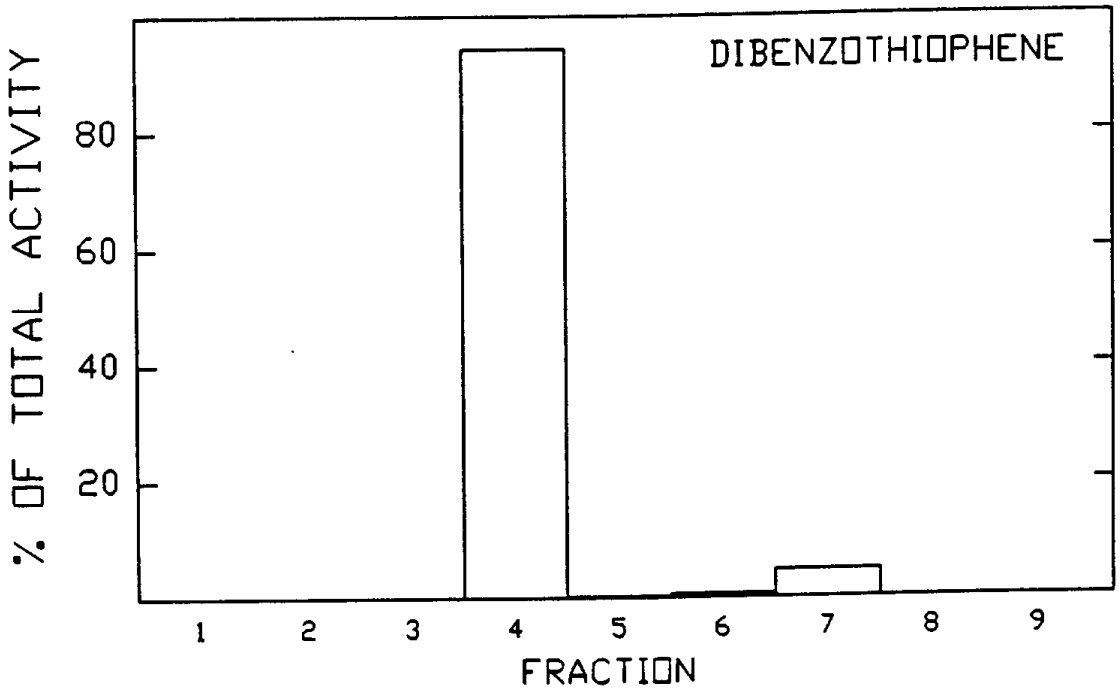


Figure 17. HPLC mutagram from the simulated atmospheric reaction of dibenzothiophene (ITC 2054). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the dibenzothiophene chamber reaction was 158,867 revertants.

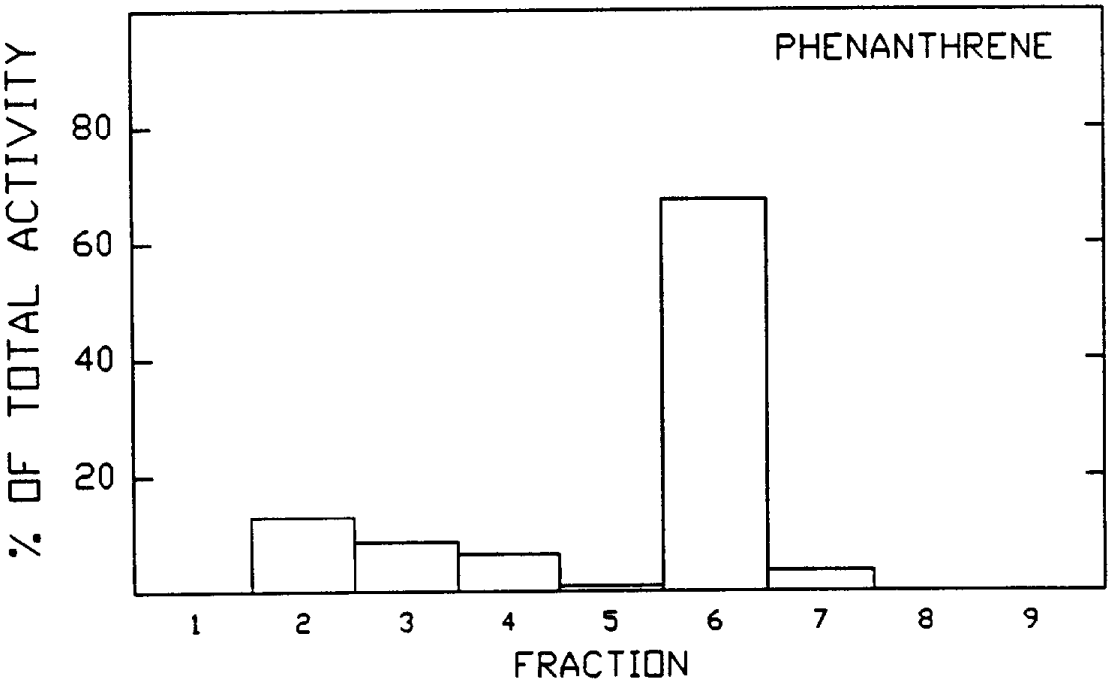


Figure 18. HPLC mutagram from the simulated atmospheric reaction of phenanthrene (ITC 1649). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the phenanthrene chamber reaction was 340,740 revertants.

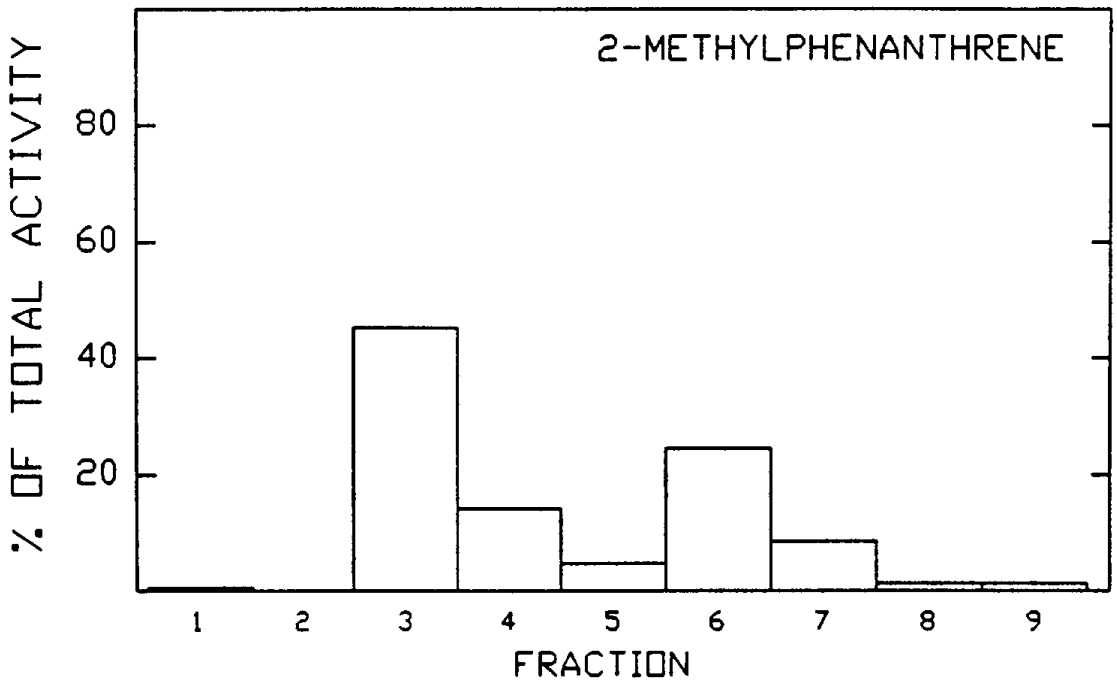


Figure 19. HPLC mutagram from the simulated atmospheric reaction of 2-methylphenanthrene (ITC 2172). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the 2-methylphenanthrene chamber reaction was 17,895 revertants.

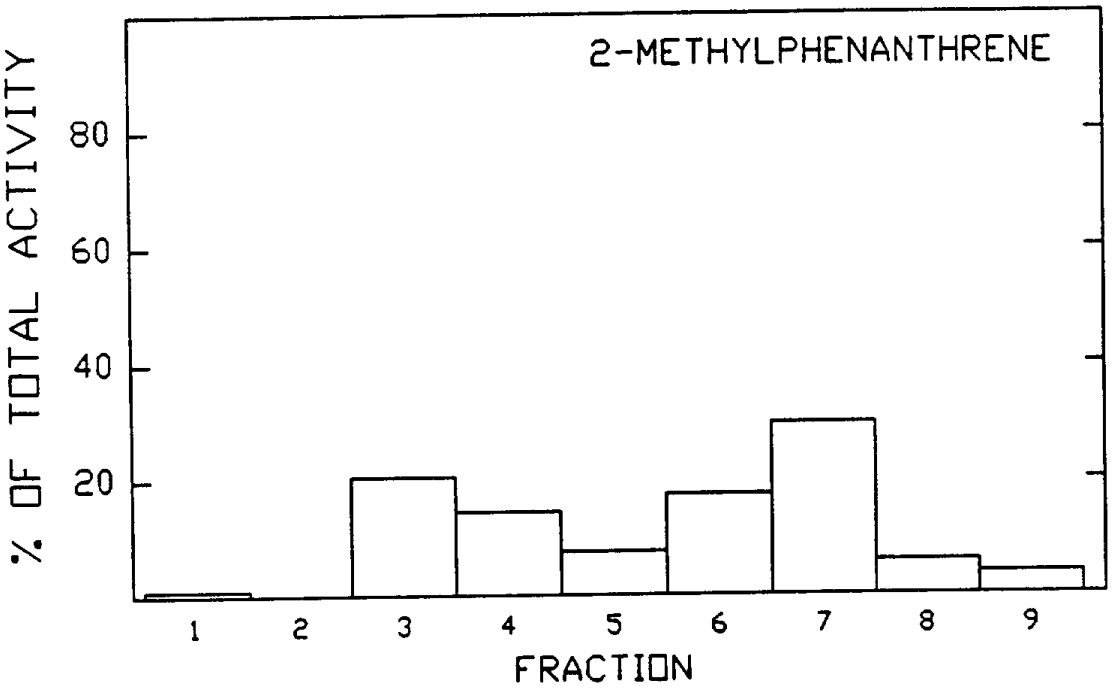


Figure 20. HPLC mutagram from the simulated atmospheric reaction of 2-methylphenanthrene (ITC 2173). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the 2-methylphenanthrene chamber reaction was 9,767 revertants.

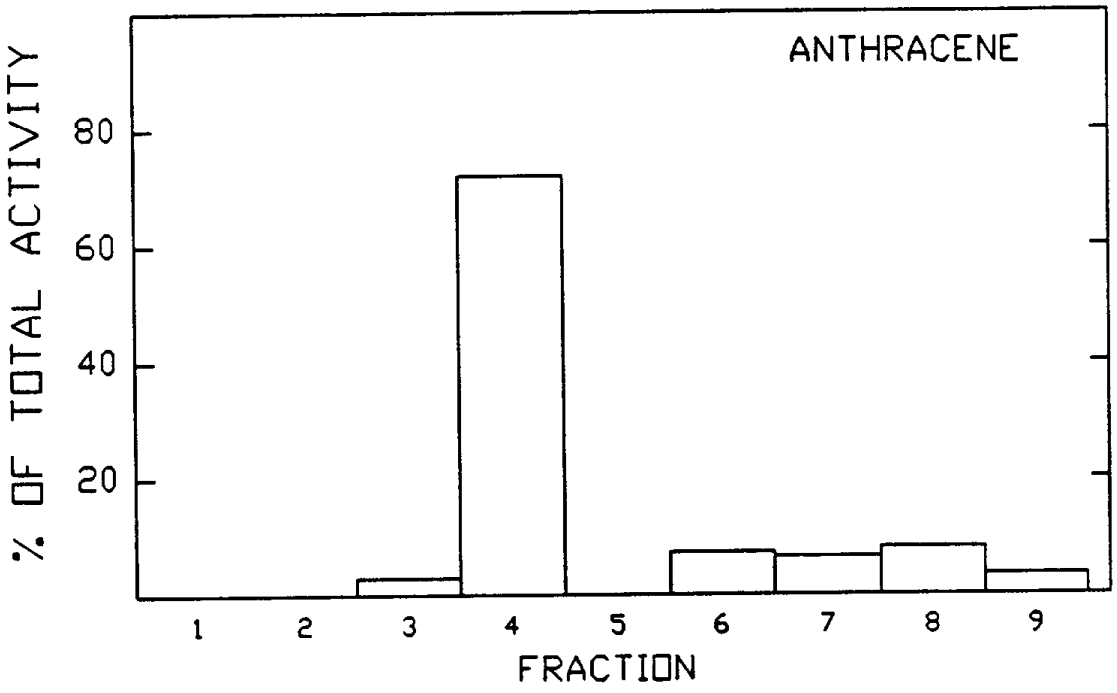


Figure 21. HPLC mutagram from the simulated atmospheric reaction of anthracene (ITC 2265). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the anthracene chamber reaction was 2,500 revertants.

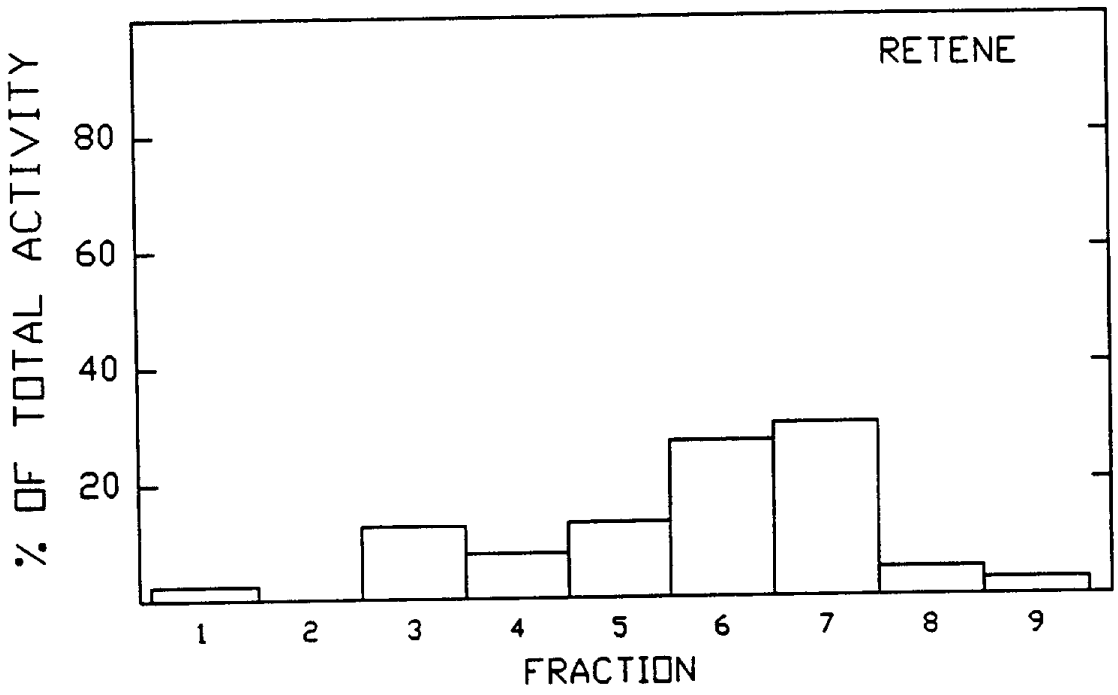


Figure 22. HPLC mutagram from the simulated atmospheric reaction of retene (ITC 2260). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the retene chamber reaction was 7,050 revertants.

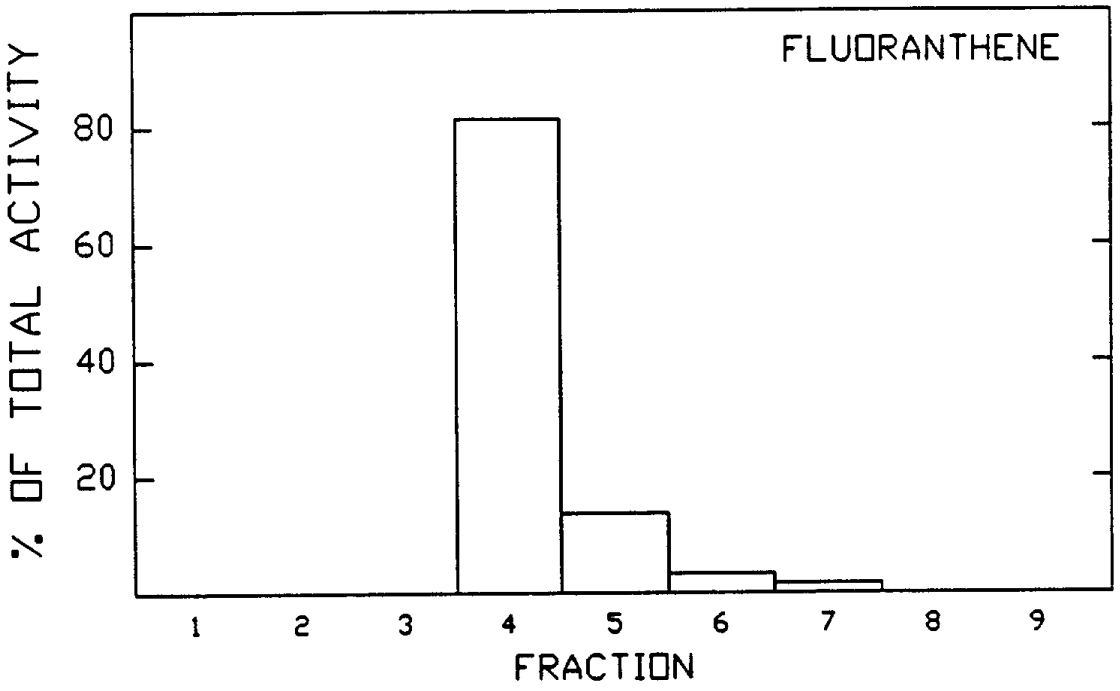


Figure 23. HPLC mutagram from the simulated atmospheric reaction of fluoranthene (ITC 2118). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the fluoranthene chamber reaction was 114,390 revertants.

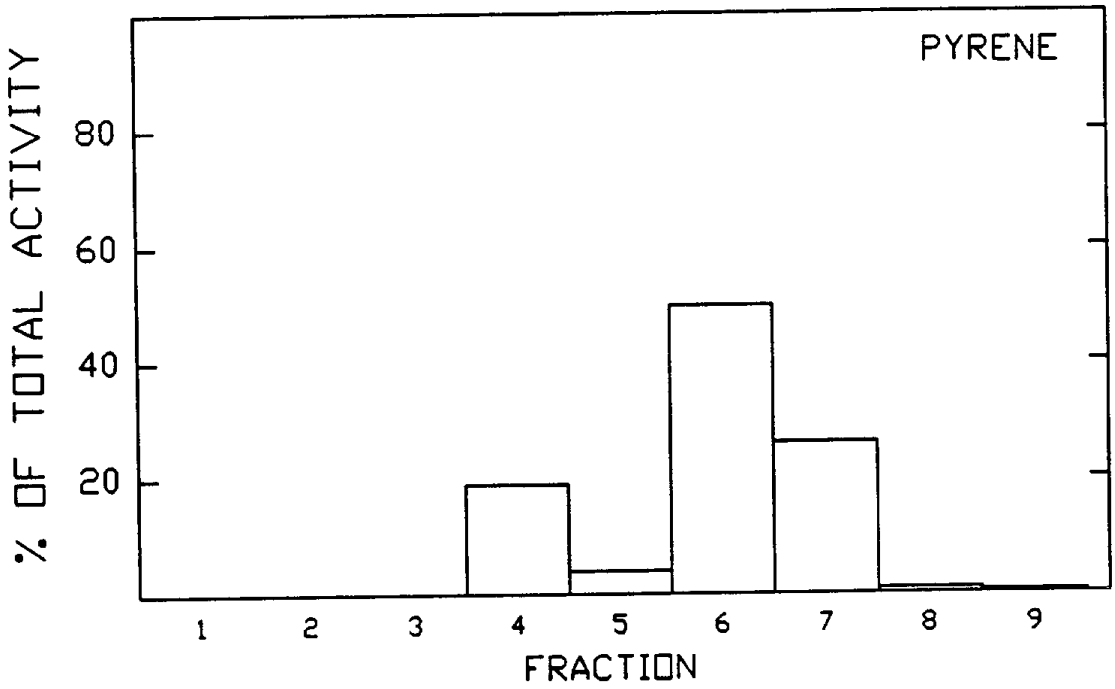


Figure 24. HPLC mutagram from the simulated atmospheric reaction of pyrene (ITC 2061). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the pyrene chamber reaction was 84,420 revertants.

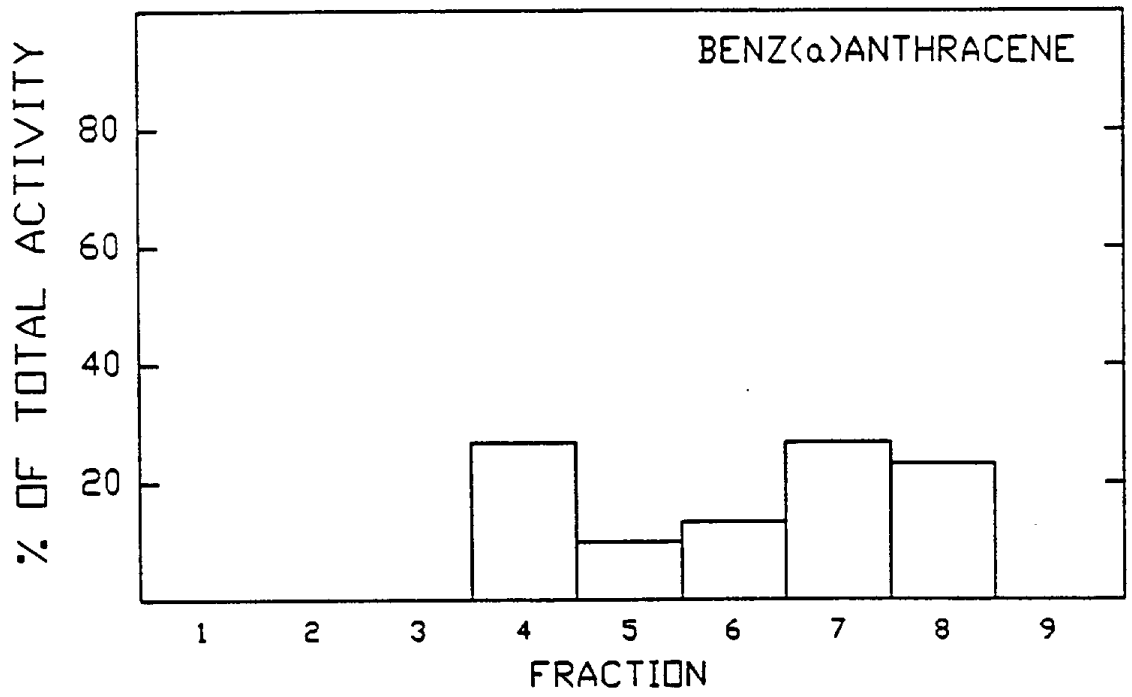


Figure 26. HPLC mutagram from the simulated atmospheric reaction of benz(a)anthracene (ITC 2271). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the benz(a)anthracene chamber reaction was 1,120 revertants.

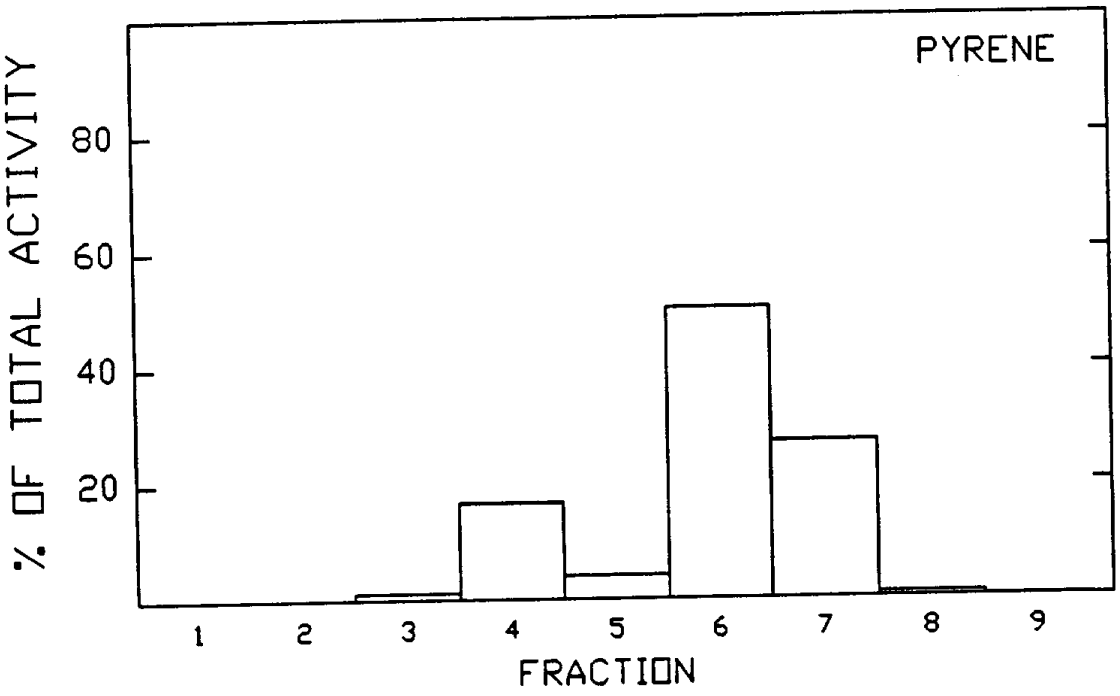


Figure 25. HPLC mutagram from the simulated atmospheric reaction of pyrene (ITC 2127-2131; composited). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the pyrene chamber reaction was 155,680 revertants.

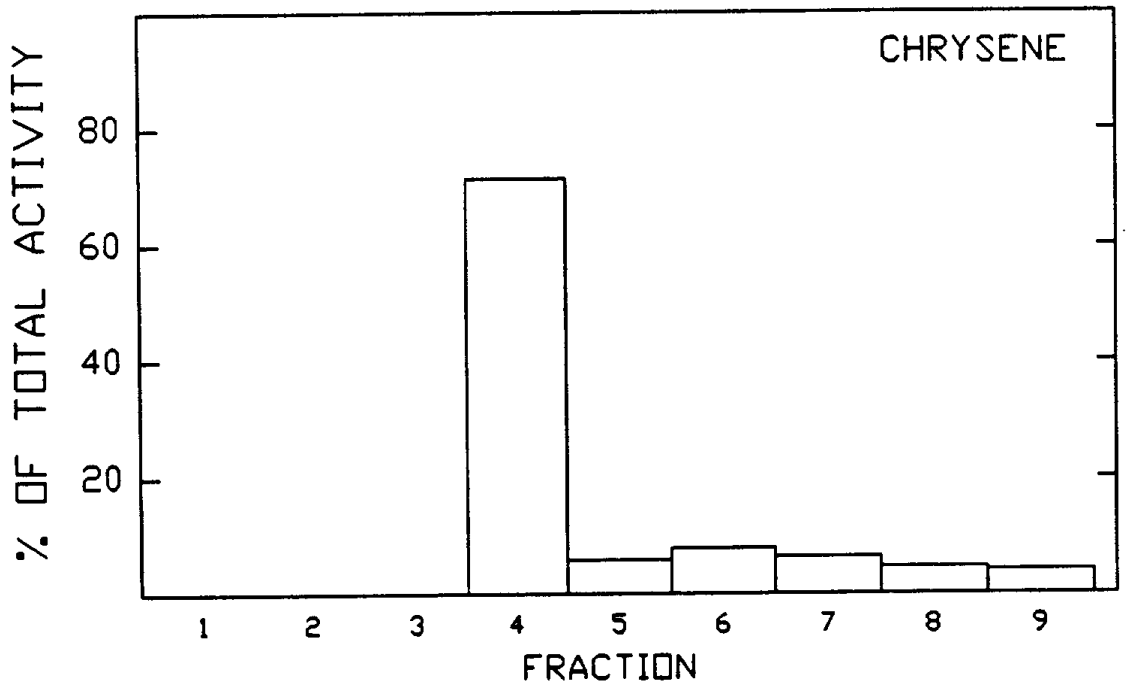


Figure 27. HPLC mutagram from the simulated atmospheric reaction of chrysene (ITC 2274-2276; composited). The plotted mutagenicity values have been normalized to the sum of the individual fractions. The total activity recovered from the chrysene chamber reaction was 2,053 revertants.

B. Chemical Analyses Results

Chemical analyses using GC/MS were performed on selected HPLC fractions of the reaction products of 1-methylnaphthalene, 2-methylnaphthalene, acenaphthene, acenaphthylene, dibenzothiophene, 2-methylphenanthrene, fluoranthene and pyrene. No chemical analyses were conducted on the products of the biphenyl, anthracene, retene, benz[*a*]anthracene and chrysene reaction products.

1. Mutagens in HPLC Fractions #4 and #3: Nitroarenes. We have previously reported that the most mutagenic HPLC fraction of the products from the simulated atmospheric reaction of naphthalene (Figure 10) contains both 1- and 2-nitronaphthalene and 1-hydroxy-2-nitronaphthalene (Arey *et al.*, 1992). 1-Hydroxy-2-nitronaphthalene has been tested in the standard Ames assay and the Kado microsuspension assay and found to be inactive (Atkinson *et al.*, 1991). Although 1- and 2-nitronaphthalene were reported to be only weak mutagens toward TA98 in the standard Ames assay, both these compounds showed significant activity when tested with the microsuspension modification (Arey *et al.*, 1992). By quantifying the nitronaphthalenes, it was estimated the ~90% of the activity of fraction #4 can be ascribed to the nitronaphthalenes with the majority of the activity being from 2-nitronaphthalene.

1-Methylnaphthalene again shows the most mutagenic activity in HPLC fraction #4 (see Figure 11). GC/MS analyses of fractions #3, #4 and #5 showed that methylnitronaphthalenes were present mainly in fraction #4 (see Figure 28). The specific 1-methylnitronaphthalene (1MxNN) isomers present were identified on the basis of their mass spectra (Arey and Zielinska, 1989). The isomers formed in order of their abundance were: 1M5NN > 1M4NN > 1M7NN > 1M2NN.

2-Methylnaphthalene showed activity in fractions #3 and #4 (see Figure 12) and the 2-methylnitronaphthalenes observed were distrubuted between these two fractions (see Figure 29). The least polar 2MxNN isomer, 2-methyl-1-nitronaphthalene, eluted entirely in fraction #3, while 2M7NN and 2M6NN eluted entirely in fraction #4. The other isomers were distributed between fractions #3 and #4 as follows (% in fraction #3: % in fraction #4): 2M8NN (39:61), 2M4NN (51:49), 2M5NN (11:89). Traces of 2M3NN (co-eluting with 2M4NN) were observed and, therefore, all seven possible 2-methylnitronaphthalene isomers were formed in the simulated atmospheric reaction. The small peak at 24.5 min in HPLC fraction #4 (Figure 29) is an isomer

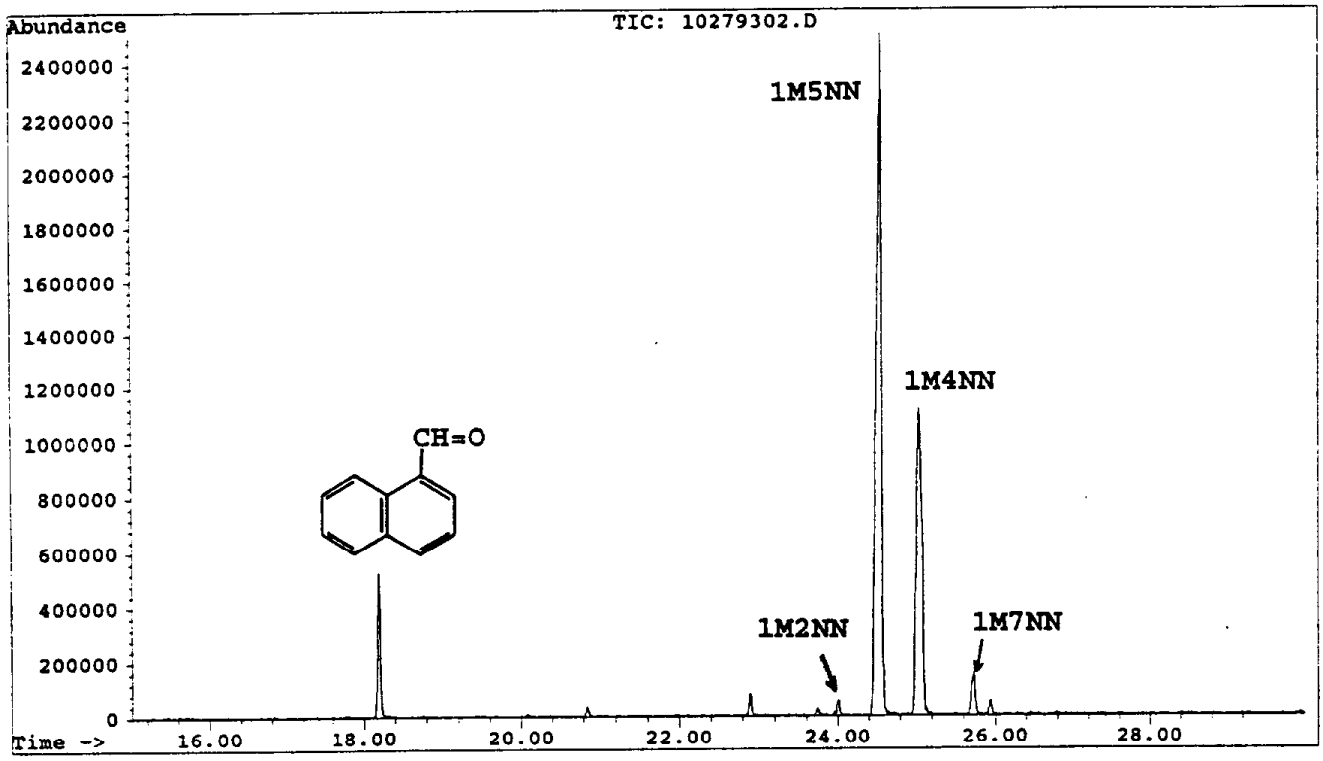


Figure 28. GC/MS total ion chromatogram (TIC) of HPLC fraction #4 from the simulated atmospheric reaction of 1-methylnaphthalene.

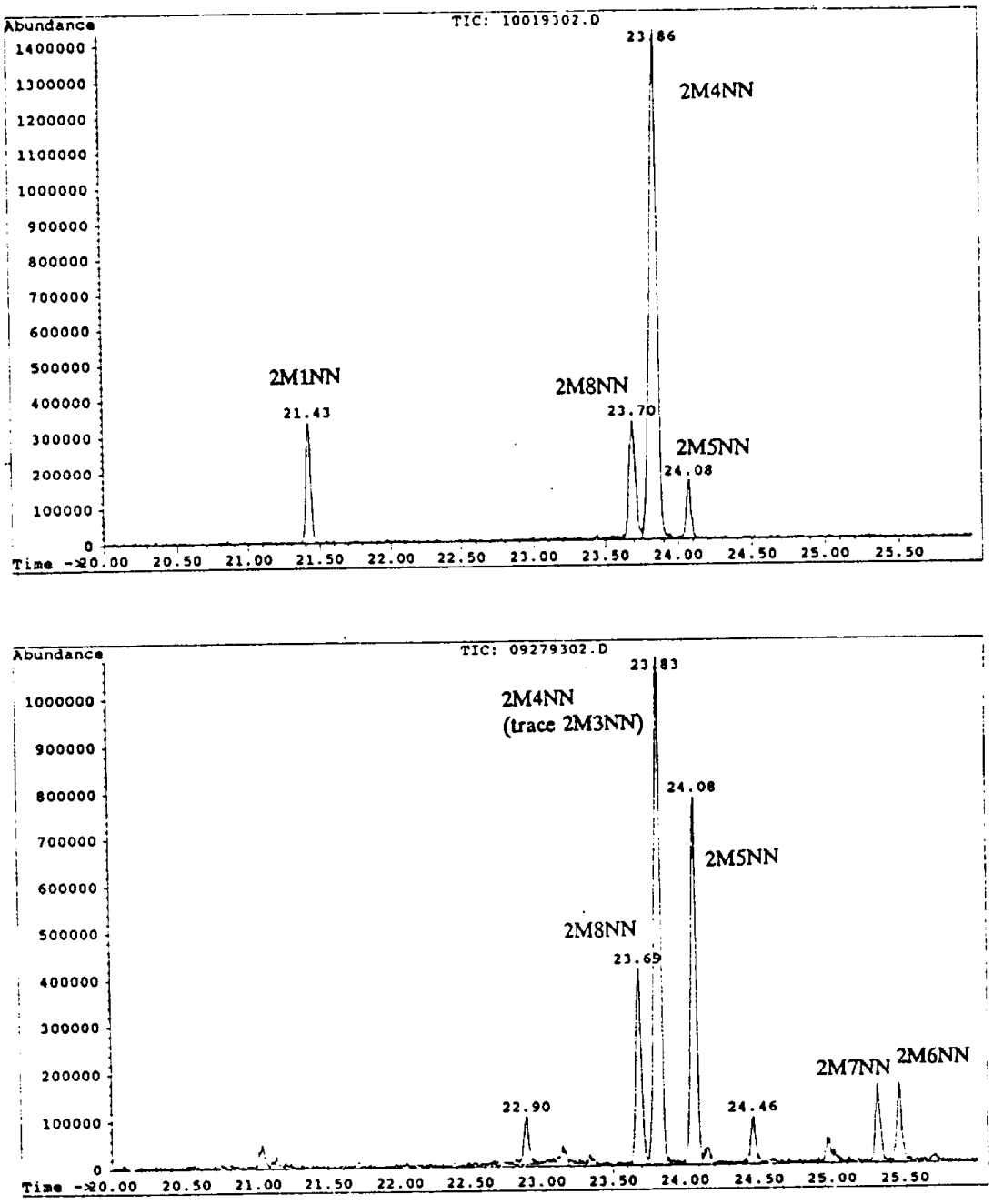


Figure 29. GC/MS TICs of HPLC fractions #3 and #4 from the simulated atmospheric reaction of 2-methylnaphthalene, showing that all seven possible 2-methylnitronaphthalenes are formed.

of 1-methylnitronaphthalene, presumably due to a small 1-methylnaphthalene impurity in the 2-methylnaphthalene introduced into the chamber.

GC/MS analysis of fraction #4 of acenaphthene (see mutagram, Figure 13) showed the presence of two nitroacenaphthene isomers identified on the basis of their spectra (Atkinson *et al.*. 1988) as 3- and 5-nitroacenaphthene, with 5-nitroacenaphthene being the more abundant isomer. In contrast to the methylnaphthalenes and acenaphthene, acenaphthylene showed significantly more activity in HPLC fractions #6 and #7 than in fraction #4 (see Figure 14).

We have previously shown 3-nitrobiphenyl to be formed from the OH radical-initiated reaction of biphenyl with an approximately 5% yield (Arey *et al.*, 1989b). The activity we measured for 3-nitrobiphenyl with the microsuspension modification of the Ames assay was 26 revertants $nmole^{-1}$. The activity in fraction #3 of the biphenyl reaction products (see Figure 15) would be consistent with the presence of 3-nitrobiphenyl, but no chemical analysis was performed.

The chemical analysis of the fluorene reaction products was reported previously (Atkinson *et al.*, 1991). Mutagenic nitrofluorenes were reported in fraction #4, and the presence of hydroxynitrofluorenes in fractions #5, #6 and #7 again suggests that hydroxylated nitro-PAH may generally be weaker mutagens than the nitro-PAH (see mutagram, Figure 16).

HPLC fraction #4 of the dibenzothiophene reaction products accounted for 94% of the total mutagenic activity (see Figure 17). GC/MS analysis of fraction #4 showed the presence of two nitrodibenzothiophene isomers (Figure 30). 2-Nitrodibenzothiophene was identified by matching the GC retention time and mass spectrum with those of a standard compound, the only nitrodibenzothiophene isomer commercially available. The estimated yields of the nitroderivatives from the simulated atmospheric reaction of dibenzothiophene were: x-nitro-dibenzothiophene, 1.5%, and 2-nitrodibenzothiophene, 0.08%.

The 2-methylphenanthrene reactions (ITC 2172 and ITC 2173) gave qualitatively similar mutagrams (see Figures 19 and 20) with very low activity in fractions #1, #2, #5, #8 and #9, and with activity in fraction #3 (and less activity in fraction #4). Nitromethylphenanthrenes were tentatively identified in HPLC fraction #3 of ITC 2172. It appears that, as seen with 2-methyl-naphthalene (see Figure 29), the presence of the methyl substituent group decreases the molecule's polarity and certain methylnitroarenes may elute mainly in HPLC fraction #3.

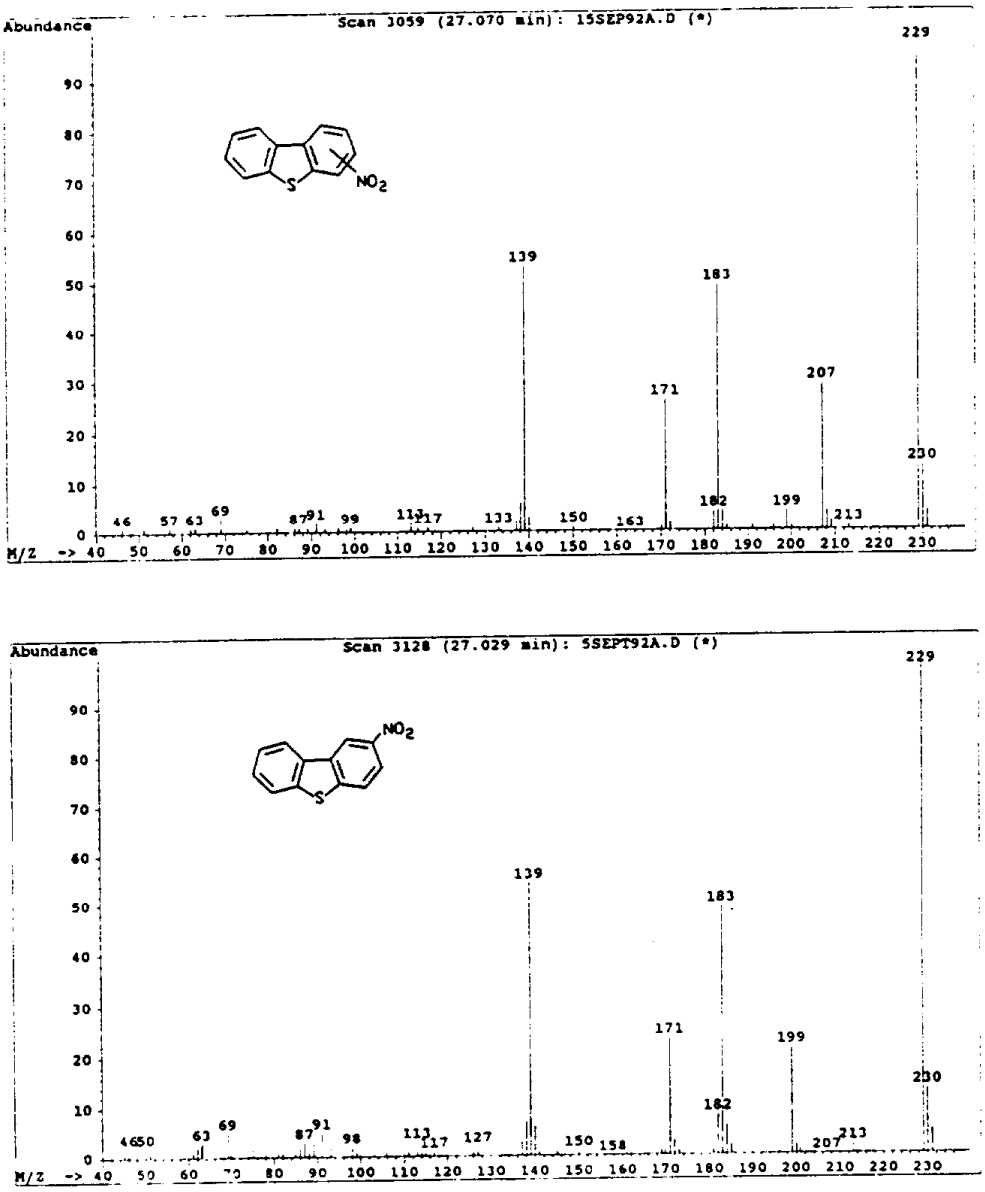


Figure 30. Mass spectra of x-nitrodibenzothiophene (top) and 2-nitrodibenzothiophene (lower). The major isomer observed in HPLC fraction #4 of the dibenzothiophene reaction products was the x-nitrodibenzothiophene isomer (the ion at m/z 207 in this spectrum is likely a column background ion).

The majority (~ 80%) of the mutagenic activity of the fluoranthene simulated atmospheric reaction (see Figure 23) resides in HPLC fraction #4. 2-Nitrofluoranthene was the sole nitroarene observed in significant yield in this fraction (see Figure 31, which also includes for comparison a separation of all eight nitrofluoranthene and nitropyrene isomers). We have previously reported (Atkinson *et al.*, 1990a) the formation 2-, 7- and 8- nitrofluoranthene with yields of 3%, 1% and 0.3%, respectively, from the OH radical-initiated reaction of gas-phase fluoranthene in the presence of NO_x and the formation of only 2-nitrofluoranthene with a formation yield of 24% from the NO_3 radical-initiated reaction. Since the long irradiation times used in these more recent experiments are expected to result in the conversion of NO to NO_2 and the formation of O_3 and NO_3 radicals during the 10 min irradiation, it is not surprising that the only nitrofluoranthene isomer observed in significant yield was 2-nitrofluoranthene. In addition to the high yield of 2-nitrofluoranthene from the NO_3 radical-initiated reaction, we have noted (Atkinson *et al.*, 1990a) that the nitrofluoranthenes photolyze under the lighting conditions of our environmental chamber. Therefore, the 7- and 8-nitrofluoranthenes expected to be formed early in the reaction may have photolyzed during the 10 min irradiation time, leaving mainly the NO_3 reaction product, 2-nitrofluoranthene.

The simulated atmospheric reactions of pyrene resulted in mutagrams (see Figures 24 and 25) with the most activity in HPLC fraction #6. Selected ion monitoring (SIM) analysis of HPLC fraction #4 of ITC 2061 showed the presence of all three nitropyrene isomers (see Figure 32). There was insufficient material from a single pyrene reaction to allow compound identification in the full scanning mode, therefore, pyrene reactions ITC 2127 through 2131 were combined, and bioassay and chemical analyses were done on the composited HPLC fractions.

2. Lactones. We previously reported the presence of 6*H*-dibenzo[*b*,*d*]pyran-6-one from the simulated atmospheric reaction of phenanthrene (Atkinson *et al.*, 1991). The pyrene lactone, tentatively identified as 5*H*-phenanthro[*4,5-bcd*]pyran-5-one, was found in HPLC fraction #5 of the pyrene simulated atmospheric reactions (see Figure 33). Two methylphenanthrene lactone isomers (as might be expected due to the asymmetry introduced by the methyl substitution of phenanthrene) were tentatively identified in HPLC fractions of the first 2-methylphenanthrene reaction, however, their distribution among several fractions was puzzling, and chemical analysis of the second reaction (ITC 2173) was not conducted. The PAH lactones do not appear to be

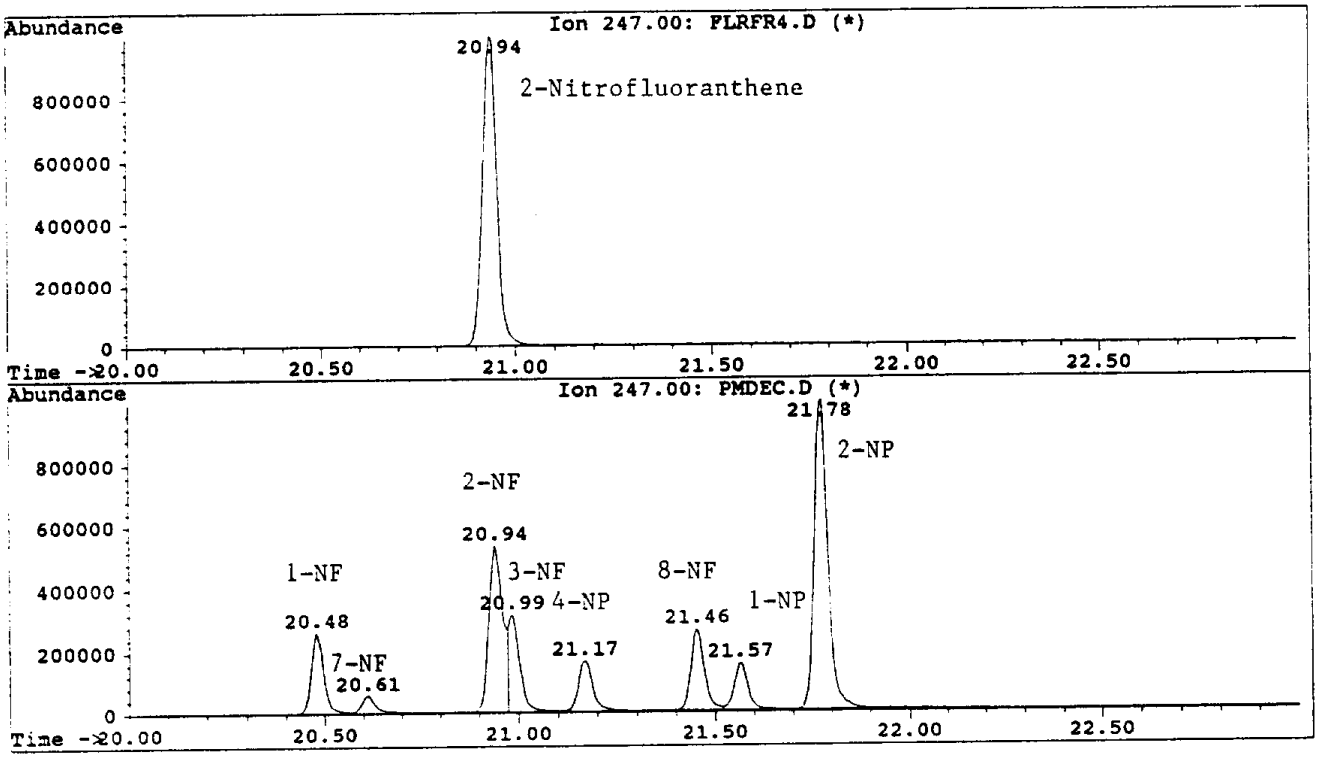


Figure 31. Ion chromatograms for the molecular ion (m/z 247) of the nitrofluoranthenes (NF) and nitropyrenes (NP). The upper ion chromatogram is from the GC/MS selected ion monitoring (SIM) analysis of HPLC fraction #4 of the fluoranthene reaction products. The lower ion chromatogram shows a separation of all eight NF and NP isomers.

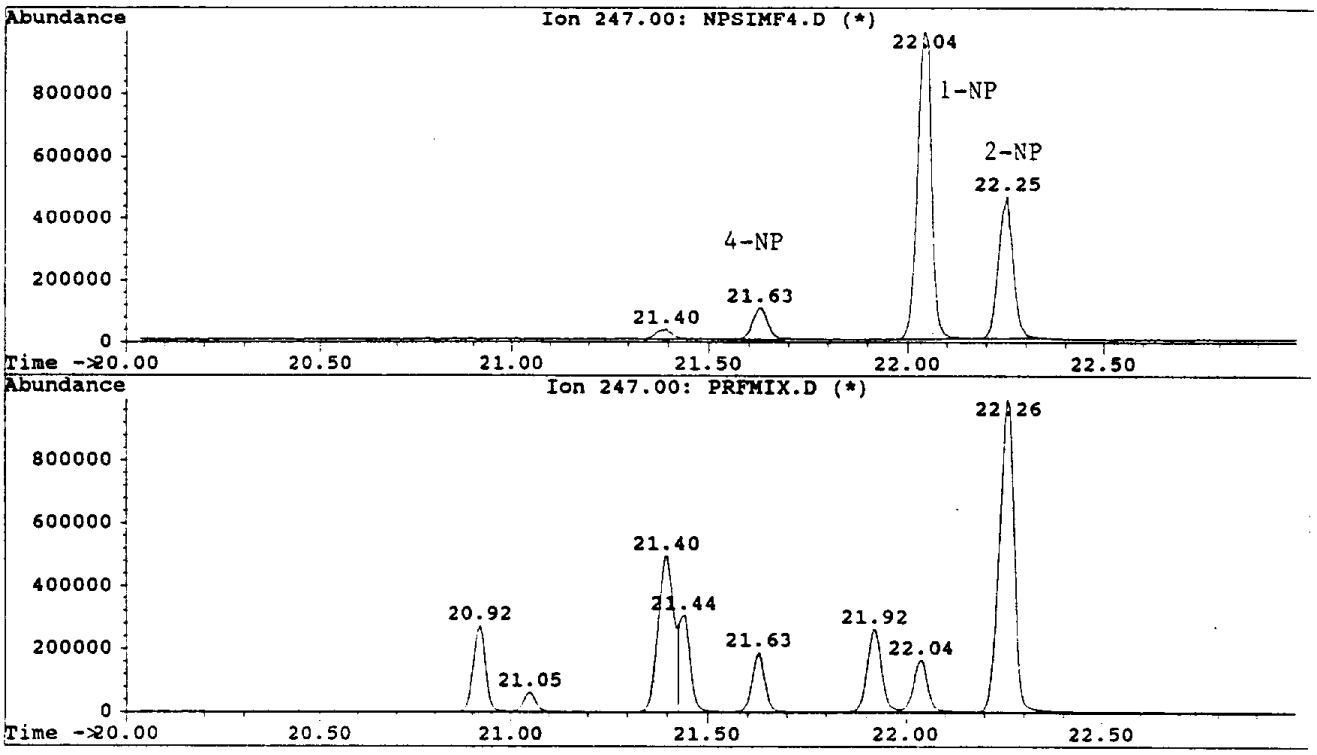


Figure 32. Ion chromatograms for the molecular ion (m/z 247) of the nitrofluoranthenes (NF) and nitropyrenes (NP). The upper ion chromatogram is from the GC/MS SIM analysis of HPLC fraction #4 of the pyrene reaction products. The lower ion chromatogram shows a separation of all eight NF and NP isomers. Note that the retention times are slightly different from those on Figure 31. The small peak at 21.4 min is 2-NF, probably resulting from a small fluoranthene impurity in our pyrene starting material.

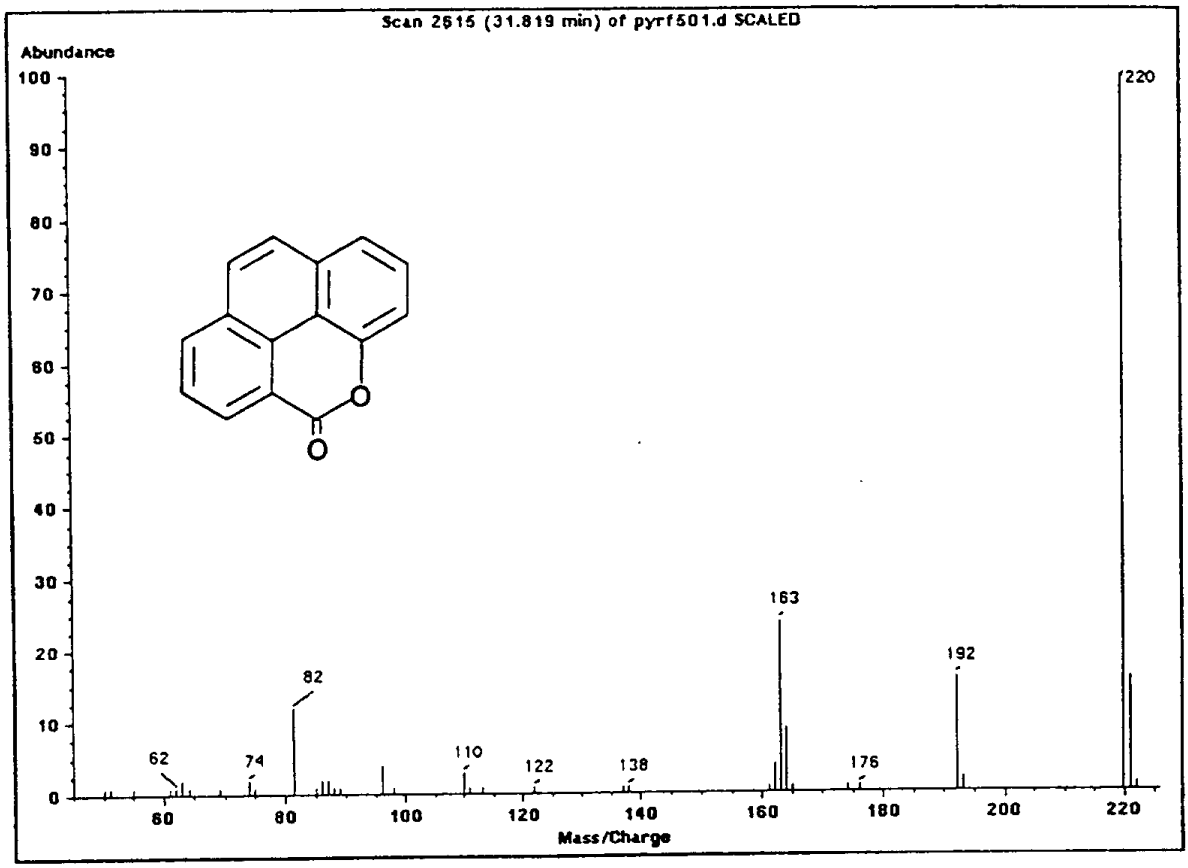


Figure 33. Mass spectrum of the pyrene lactone, tentatively identified as 5*H*-phenanthro[*4,5-bcd*]pyran-5-one.

mutagenic in themselves, but as noted below, the nitro-PAH lactones appear to be strong mutagens.

3. Mutagens in HPLC Fractions #6 and #7: Nitro-PAH Lactones. We have previously reported (Atkinson *et al.*, 1991; Arey *et al.*, 1992; Helmig *et al.*, 1992a; Helmig and Arey, 1992) the presence of 2- and 4-nitrodibenzopyranone in highly mutagenic subfractions of HPLC fraction #6 of the phenanthrene reaction products. As seen from the mutagram of the phenanthrene reaction (Figure 18), the majority of the activity resides in this fraction. Mutagenicity testing of a 2-nitro-6*H*-dibenzo[*b,d*]pyran-6-one standard showed that this isomer alone could account for the observed activity.

As noted above, several pyrene reactions were composited to allow full scanning GC/MS analysis of HPLC fraction #6. It was decided to further subfractionate HPLC fraction #6, as had been done with the phenanthrene products. Each subfraction was 1 min and over 80% of the activity occurred in two subfractions #6.1 and #6.3. GC/MS analysis showed the presence of two different tentatively identified nitropyrene lactones, one in each subfraction. The lower portion of Figure 34 shows the mass spectra obtained from the two very mutagenic subfractions #6.1 and #6.3. For comparison, the spectrum of 2-nitro-6*H*-dibenzo[*b,d*]pyran-6-one is also shown on the top portion of the figure. The observed molecular weights for the peaks eluting with GC retention times of 39.9 (pyrene HPLC subfraction #6.1) and 44.5 min (pyrene HPLC subfraction #6.3) are 265, consistent with these peaks being nitropyrene lactones. The similar fragmentation pattern of the two isomers to that of the nitrophenanthrene lactone, namely losses of: [M-NO], [M-NO-CO], [M-NO_2-CO], [M-C_2NO_3], and [M-C_2NO_4] suggest that two distinct, and obviously very mutagenic, nitropyrene lactones are formed in the pyrene reaction.

Although the acenaphthylene reaction also showed significant activity in HPLC fraction #6 (see Figure 14), nitrolactone species do not seem to be responsible for this activity. GC/MS analysis of fraction #6 of the acenaphthylene reaction products showed two compounds of apparent molecular weight 213. The spectra suggest nitrated compounds, perhaps nitrated epoxide or nitrated hydroxy- derivatives of acenaphthylene.

4. Other Products. HPLC fractions #3, #4 and #5 of the 1- and 2-methylnaphthalene chamber reactions were analyzed by GC/MS. As noted above, methylnitronaphthalenes were contained mainly in fraction #4 from the 1-methylnaphthalene reaction products and fractions

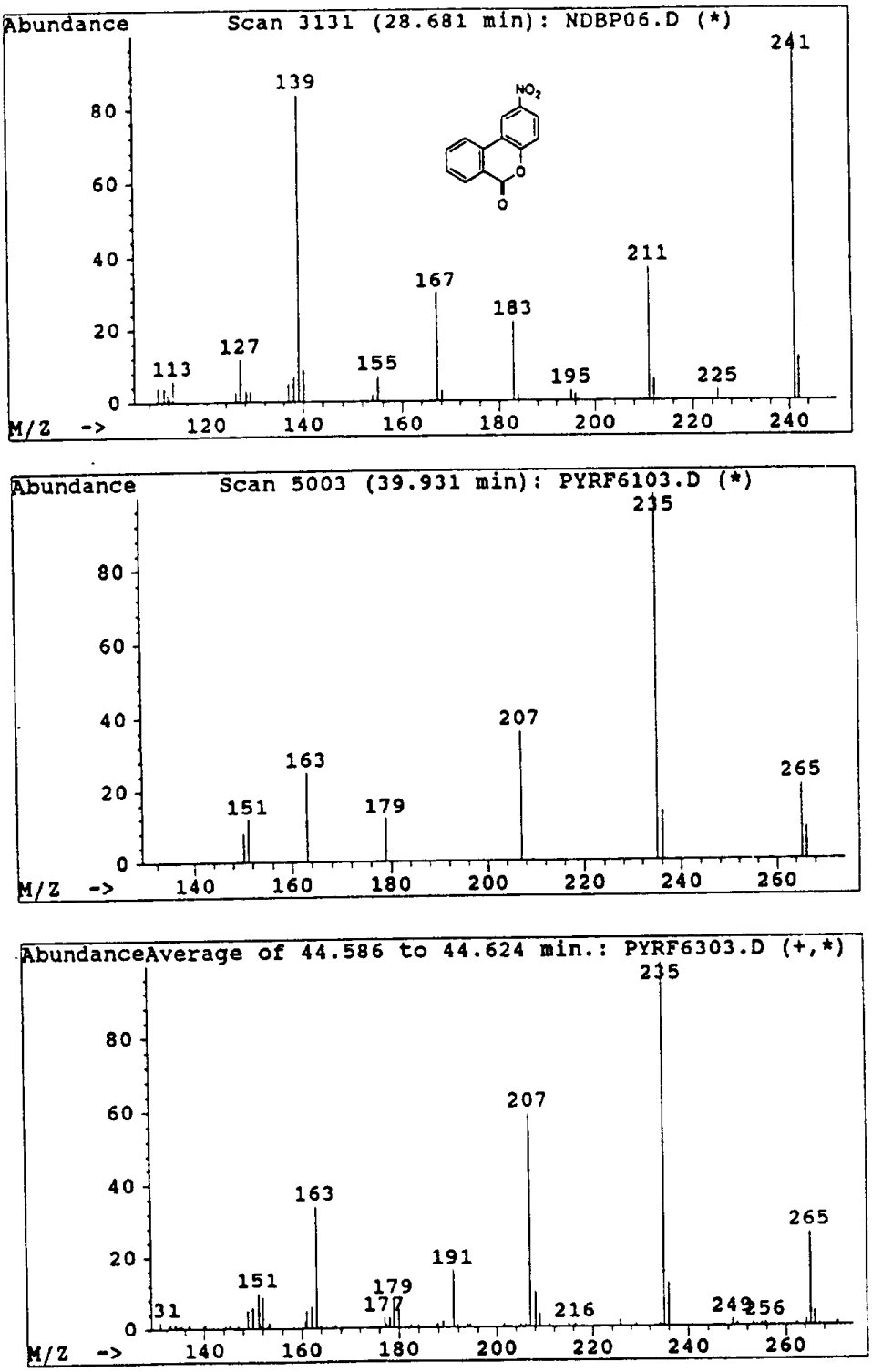
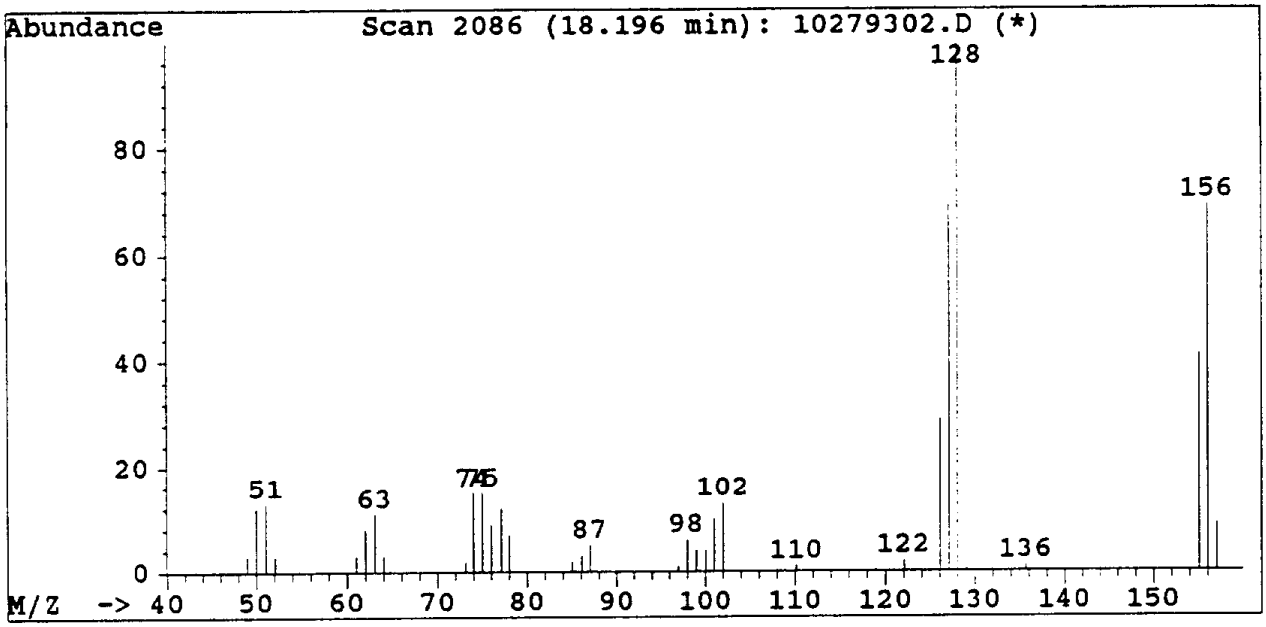


Figure 34. The top mass spectrum is that of 2-nitro-6*H*-dibenzo[*b*,*d*]pyran-6-one. The middle and lower spectra are from the GC/MS analyses of HPLC subfractions 6.1 and 6.3, respectively, of the simulated atmospheric reaction of pyrene.

#3 and #4 from the 2-methylnaphthalene reaction products. A compound tentatively identified as 1-naphthalenecarboxaldehyde (see Figure 35) was found in fractions #4 and #5 (more being in fraction #5) of the 1-methylnaphthalene reaction. A compound tentatively identified as 2-naphthalenecarboxaldehyde was present in fraction #5 of the 2-methylnaphthalene reaction (see Figure 36). A compound tentatively identified as a methylnitronaphthol (molecular weight 203) was seen in fraction #3 of the 1-methylnaphthalene reaction, while four tentatively identified methylnitronaphthols were fairly evenly distributed between fractions #4 and #5 of the 2-methylnaphthalene reaction products (see Figure 37).

Additionally several other oxygenated methylnaphthalene derivatives were found in fraction #5 from both methylnaphthalene reactions. Figure 38 shows the spectrum of one interesting compound tentatively identified as an epoxide derivative.

Although the methyl-substituted PAH are expected to react mainly by OH radical addition to the ring, the presence of naphthalenecarboxaldehydes among the reaction products of the methylnaphthalenes (Figures 35 and 36) and phenanthrenecarboxaldehyde (presumably 2-phenanthrenecarboxaldehyde) from the 2-methylphenanthrene reaction (see lower spectrum Figure 35) shows that some reaction occurs at the methyl group.



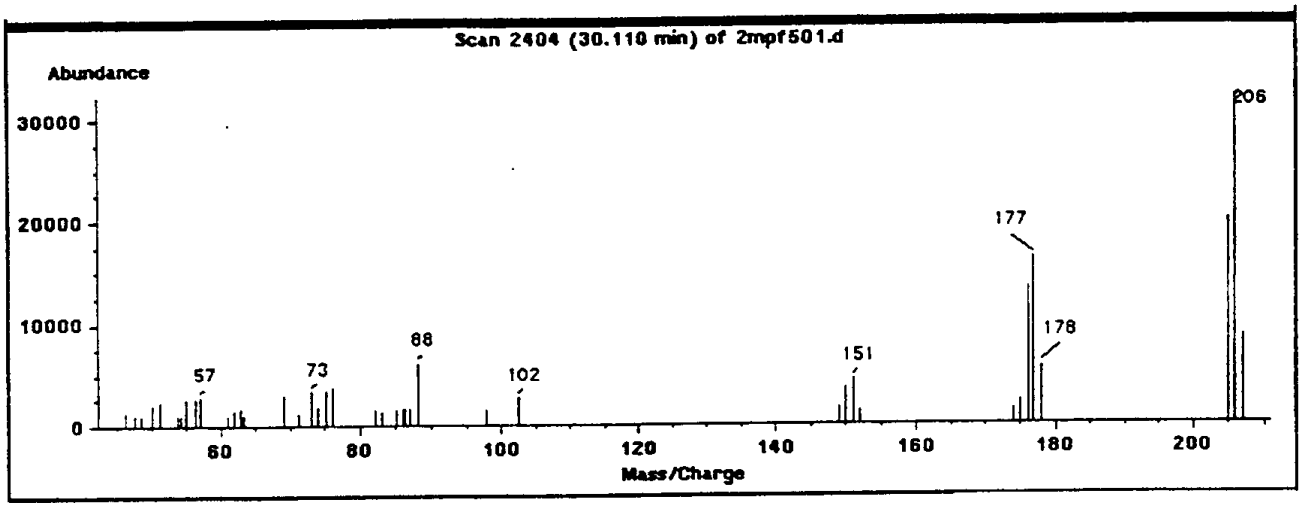


Figure 35. Mass spectra of compound eluting in HPLC fractions #4 and #5 (more present in fraction #5) of the reaction products of 1-methylnaphthalene (top) and HPLC fraction #... of the 2-methylphenanthrene reaction products. The compounds are tentatively identified as 1-naphthalene-carboxaldehyde (upper) and 2-phenanthrenecarboxaldehyde (lower).

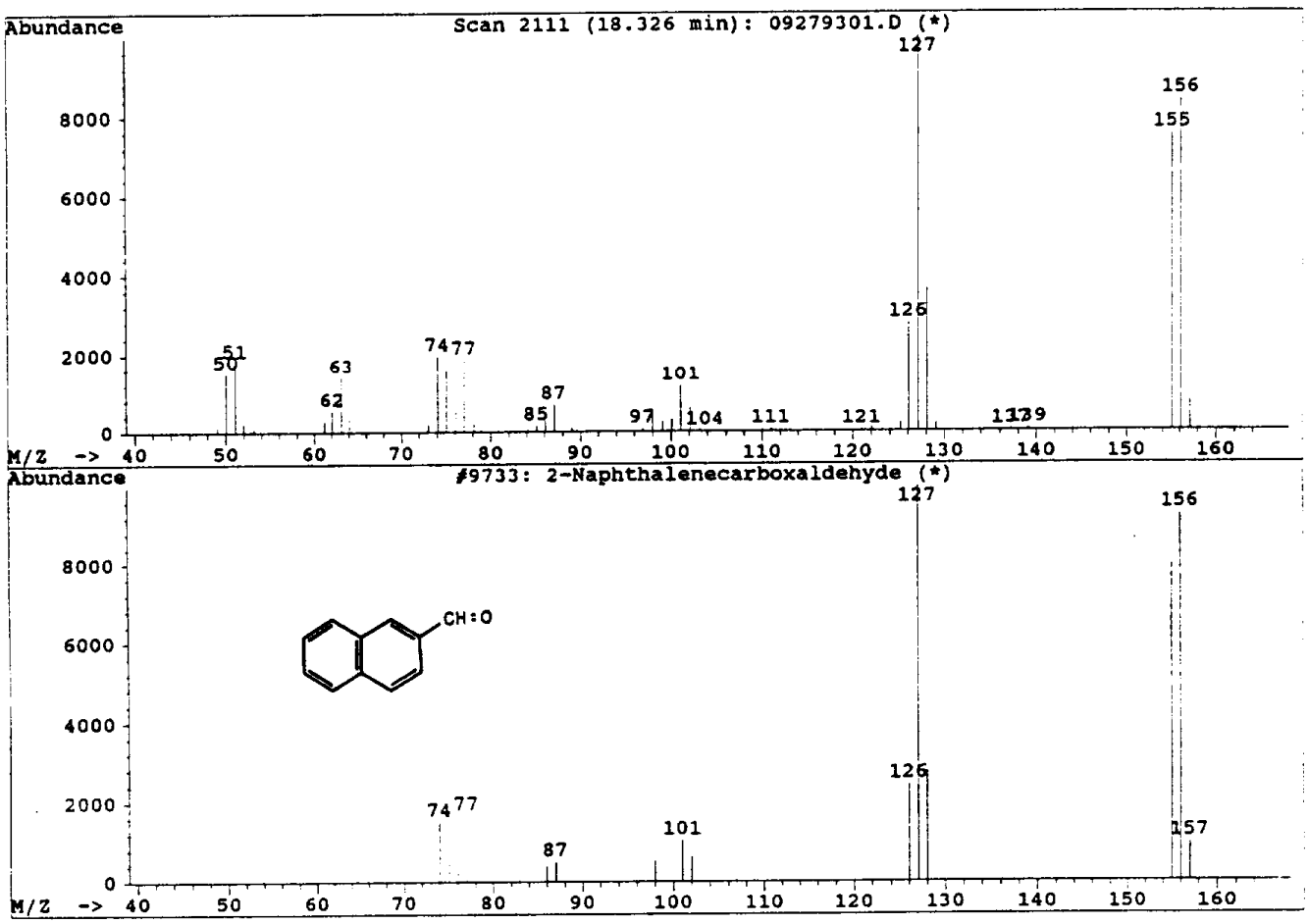


Figure 36. Mass spectra of a compound eluting in HPLC fraction #5 from the 2-methylnaphthalene reaction (upper) and tentatively identified as 2-naphthalenecarboxaldehyde. Also shown is a reference spectrum of 2-naphthalenecarboxaldehyde (lower).

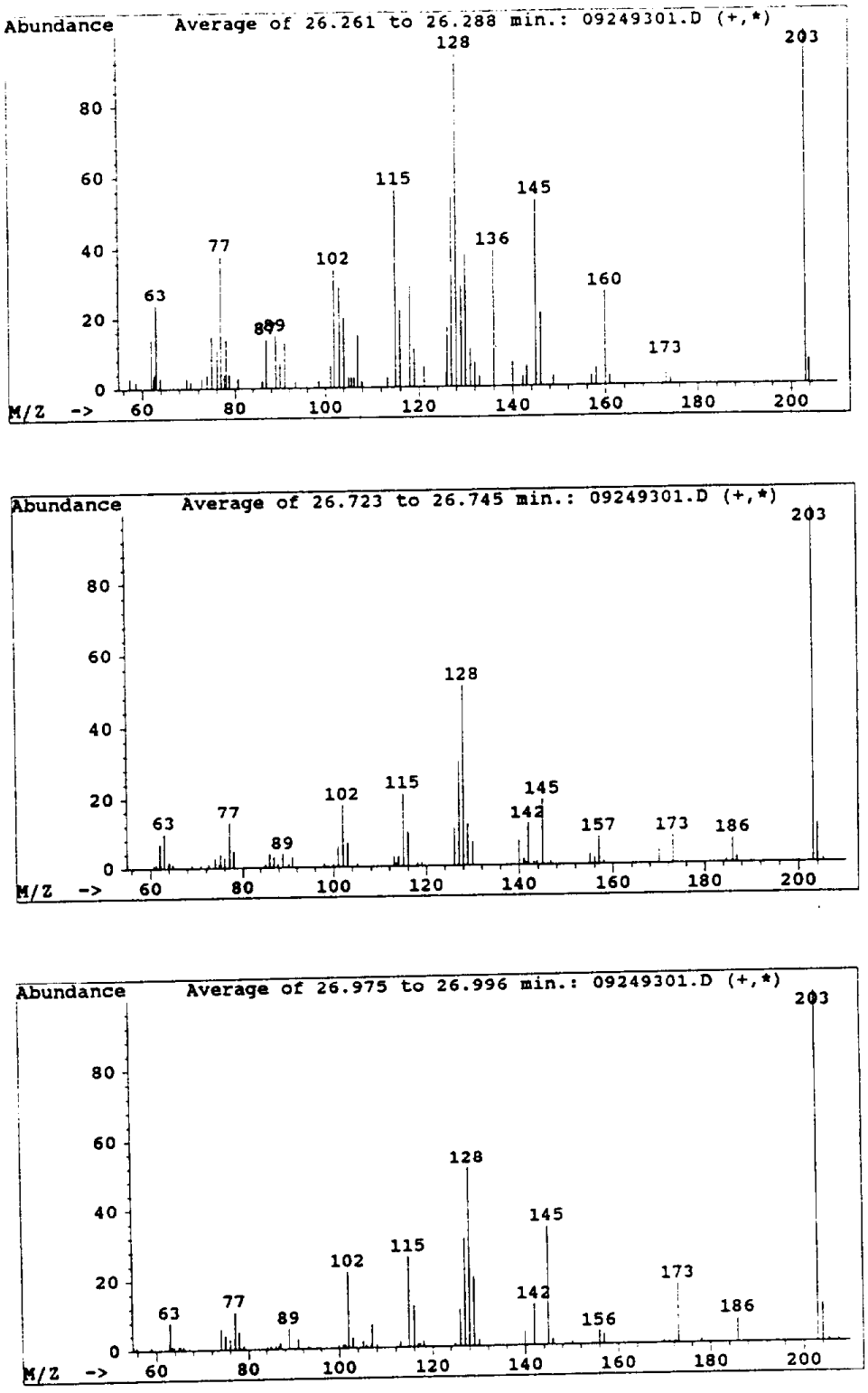
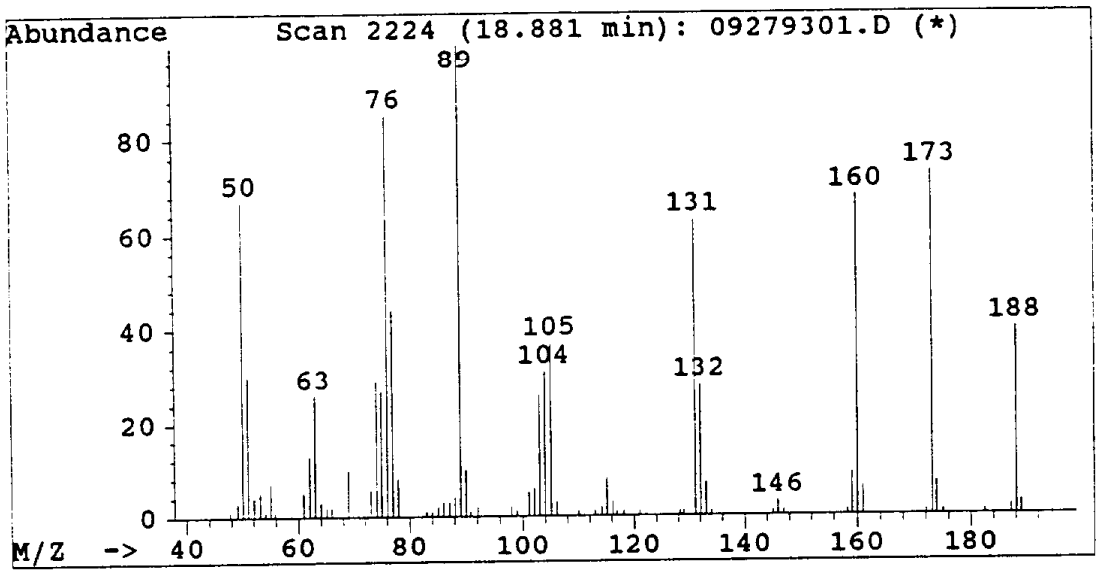


Figure 37. Mass spectra of three tentatively identified methylnitronaphthols from the simulated atmospheric reaction of 2-methylnaphthalene. These compounds were distributed between HPLC fractions #4 and #5.



188 $C_{11}H_8O_3$ 15448-59-6
Naphth[2,3-*b*]oxirene-2,7-dione, 1a,7a-dihydro-1a-methyl-

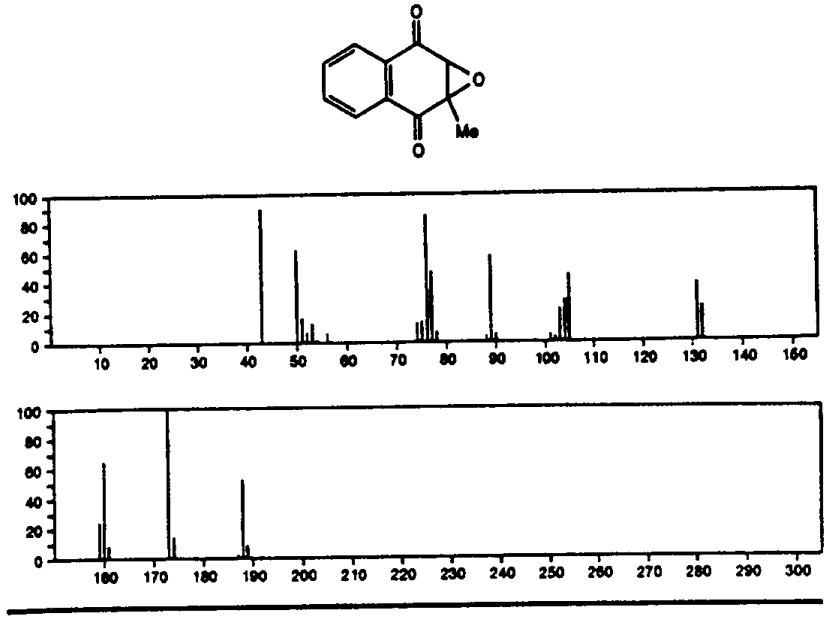


Figure 38. Mass spectrum of a compound eluting in HPLC fraction #5 from the 2-methylnaphthalene reaction (upper) and tentatively identified as 1a,7a-dihydro-1a-methylnaphth[*2,3-b*]oxirene-2,7-dione. Also shown is a reference spectrum of the epoxide (lower).

DISCUSSION

As can be seen from Tables 6-23 and Figures 10-27, the PAH studied fall into two general groups, one group of PAH forming mutagenic products primarily in our HPLC fraction #4 (or #3 in some cases), and the other group of PAH forming mutagenic products primarily in fraction #6 (and #7). The first group of PAH includes naphthalene, 1- and 2-methylnaphthalene, acenaphthene, biphenyl, fluorene, dibenzothiophene, anthracene, fluoranthene, and chrysene, and the present chemical analyses and previous product studies (Arey *et al.*, 1986, 1989b; Atkinson *et al.*, 1987, 1990a; Zielinska *et al.*, 1989b; Atkinson and Arey, 1994) suggest that these PAH form (together with other products) direct-acting mutagenic nitro-PAH which elute in fraction #4 (or, for the methyl-PAH and biphenyl, fraction #3 or fractions #3 and #4).

The second group of PAH exhibit mutagrams with the majority of the mutagenicity in the more polar fractions #6 and 7, and include acenaphthylene, phenanthrene, retene and pyrene. [2-Methylphenanthrene and benz[*a*]anthracene appear to be intermediate between these two groups of PAH, with the majority of the mutagenicity from their photooxidations appearing distributed among fractions #3, 4, 6 and 7]. Present and previous (Arey *et al.*, 1992; Helmig *et al.*, 1992a,b) product studies and chemical analyses of HPLC fractions have shown that at least two of this second group of PAH, phenanthrene and pyrene, form direct-acting mutagenic nitro-PAH lactones (for example, 2-nitrodibenzopyranone from phenanthrene) which elute in fraction #6 with the present HPLC solvent program. Interestingly, a K-region structure appears to be necessary for the formation of mutagenic nitro-PAH lactones.

The present mutagenicity data from the CH_3ONO-NO-PAH-air irradiations can be used to roughly estimate the contributions of the individual PAH (through their atmospheric photooxidations) to the direct-acting mutagenicity of extracts of samples collected from ambient air. For this assessment, the sample volumes collected from the chamber and the differences in the PAH concentrations in the chamber *versus* ambient air have been taken into account. Several of the PAH (the less volatile ones) were sprayed into the chamber in mg quantities, far in excess of the amount required to bring the gas-phase to saturation. The similar PAH concentrations before and after the irradiations (Table 5) suggests that fairly rapid volatilization occurred maintaining the gas-phase concentration. The idea of spraying the PAH was to

disperse it as much as possible onto the chamber walls, providing maximum surface area for volatilization. For the PAH introduced into the chamber as gases (by flowing N_2 gas through the solid PAH), the amounts reacted are given by,

$$[\text{PAH}]\ \text{reacted} = [\text{PAH}]_{t_0} - [\text{PAH}]_t \qquad \text{(II)}$$

while for those sprayed into the chamber, with almost constant concentrations before and after the irradiations (and assuming only reaction of the PAH with the OH radical),

$$[\text{PAH}]\ \text{reacted} = k_{OH}[OH][\text{PAH}]_{av}(t_0 - t) \qquad \text{(III)}$$

where k_{OH} is the rate constant for reaction of the PAH with the OH radical, [OH] is the OH radical concentration in the chamber, and $[\text{PAH}]_{t_0}$ and $[\text{PAH}]_t$ are the PAH concentrations at times t_0 and t (i.e., before and after the irradiation). Using an OH radical reaction rate constant of 2×10^{-11} cm^3 $molecule^{-1}$ s^{-1} (reasonably typical of the PAH; see, for example, Atkinson and Arey, 1994), an OH radical concentration of 1×10^8 molecule cm^{-3} (see above and Atkinson *et al.*, 1989), then for a 10 min reaction time (the product of $k_{OH}[OH](t_0-t) = 1.2 \approx 1$).

$$[\text{PAH}]\ \text{reacted} \sim [\text{PAH}]_{av} \qquad \text{(IV)}$$

Furthermore, since a relatively large fraction of most of the "gas-phase" PAH (i.e., those introduced by passing a stream of gas through the solid PAH) reacted during the 10 min irradiation time, to a reasonable approximation the initial PAH concentration can be used as the [PAH] reacted and as the concentration which produced the mutagenicity measured in the chamber PUF sample. Knowing the mutagenic activity resulting from a given [PAH] reacted, the mutagenicity formed in the atmosphere from the individual PAH can be calculated from its ambient concentration.

We have previously measured the ambient concentrations of all of the PAH studied here (except 2-methylphenanthrene) in Glendora, CA, during August 1986 (Arey *et al.*, 1989b), and

the nighttime concentrations, averaged over a 9-day period, are given in Table 24 in ppt. Nighttime concentrations are used since they are most comparable to the pre-reaction chamber PAH concentrations. The estimated contribution (revertants m^{-3}) of the individual PAH to the ambient air mutagenicity in HPLC fraction #i is given by,

$$\text{Ambient mutagenicity, Fraction \#i} = (\text{Chamber mutagenicity, Fraction \#i}) \times \{[\text{PAH}]_{air}/([\text{PAH}]_{chamber}\ V)\} \quad (V)$$

where V is the volume sampled from the chamber (in m^3). The estimated ambient atmospheric mutagenicities in the various HPLC fractions are given for all of the PAH studied in Table 25, together with the estimated overall ambient air mutagenicity from that PAH.

Of course, such calculations assume that the chamber experiments accurately simulate the ambient atmosphere in the photooxidation of the PAH, and we have already shown that this is not strictly the case. Thus, for phenanthrene, we observed the yield of 2-nitrodibenzopyranone (the mutagenic nitro-PAH lactone formed from phenanthrene) was $\sim 0.1\%$ in the chamber experiments, but ambient atmospheric measurements indicate a yield of $\sim 1\%$, approximately an order of magnitude higher (Helmig *et al.*, 1992b). This particular discrepancy probably arises because 2-nitrodibenzopyranone is not a primary product of the photooxidation of phenanthrene (Helmig *et al.*, 1992b; Kwok *et al.*, 1994), and the chamber experiments are probably not long enough for the nitrodibenzopyranones to be formed in the same overall yield as occurs in ambient air, although it should be noted that the present irradiations are equivalent to $\sim$12-15 hours of daylight, based on the chamber and ambient air OH radical concentrations.

However, our data (Table 25) do suggest that certain of the PAH contribute more to ambient mutagenicity than do others, with naphthalene, 1- and 2-methylnaphthalene, fluorene, dibenzothiophene, phenanthrene, fluoranthene and pyrene being important contributors and acenaphthene, acenaphthylene, biphenyl, 2-methylphenanthrene, retene, benz[*a*]anthracene and chrysene not being important, at least with the sampling and testing procedure used here. The calculated mutagenicities due to PAH reaction products can be compared with ambient measurements conducted using the same sampling, extraction, HPLC fractionation and Kado bioassay as used in this work (Arey *et al.*, 1992; Harger *et al.*, 1992), although at a different

Table 24. Ambient mixing ratios of PAH in the gas-phase at Glendora, CA (August 1986). From Arey *et al.* (1989a); Atkinson *et al.* (1988).

PAH	Mixing Ratio (ppt)[a]
Naphthalene[b]	840
1-Methylnaphthalene[b]	65
2-Methylnaphthalene[b]	130
Acenaphthene[b]	4.0
Acenaphthylene[b]	2.0
Biphenyl[b]	16
Fluorene[b]	7
Dibenzothiophene[c]	0.5
Phenanthrene[b]	7
2-Methylphenanthrene[d]	≤7
Anthracene[c]	0.2
Retene[e]	<0.02
Fluoranthene[c]	0.8
Pyrene[c]	0.6
Benz(a)anthracene[e]	<0.02
Chrysene[e]	<0.1

[a]Nighttime values.
[b]From samples collected on Tenax solid adsorbent (Arey *et al.*, 1989a).
[c]From samples collected on PUF solid adsorbent (Atkinson *et al.*, 1988).
[d]Expected upper limit.
[e]Upper limit, atmospheric concentration as measured from samples collected on filters (Atkinson *et al.*, 1988).

Table 25. Contributions to ambient mutagenicity (revertants m^{-3}) estimated from chamber photooxidations of gas-phase PAH.

	HPLC Fraction No.[a]									
PAH	1	2	3	4	5	6	7	8	9	Total
Naphthalene				35	2.5	0.4	5.3	3.0	0.7	47
1-Methylnaphthalene				7.6	1.8	0.1	0.7	0.1		10
2-Methylnaphthalene			0.5	5.1						5.6
Acenaphthene				0.4			0.2			0.6
Acenaphthylene				0.1		0.3	0.3			0.7
Biphenyl			0.1							0.2
Fluorene		0.1		3.9	0.7	0.5	0.7			5.8
Dibenzothiophene				3.9			0.2			4.1
Phenanthrene		1.0	0.6	0.5	0.1	5.0	0.3			7.5
2-Methylphenanthrene[b]			0.1			0.1				0.3
Anthracene										0.2
Retene										0.003
Fluoranthene				25	4.2	0.1	0.5			30
Pyrene[c]			0.1	1.8	0.5	5.5	3.0	0.1		11
Benz(a)anthracene										0
Chrysene				0.6	0.1	0.1	0.1			0.9

[a]If no entry is given, the calculated rev m^{-3} was <0.1.
[b]Used averaged results from ITC 2172 and ITC 2173.
[c]Used results from ITC 2127-2131 (normalized).

time and place (Claremont, CA, during August 1987) than the ambient air PAH data were obtained (although still within the Los Angeles air basin). Such a comparison is made in Table 26, from which it can be seen that our chamber data predict a direct-acting ambient air mutagenicity which is 33% of that observed (at a different time and place). The predicted fraction #4 mutagenicity, due primarily to nitro-PAH, is close to that measured, while the predicted fraction #6 and #7 mutagenicities are a factor of ~ 10 lower than measured in ambient air. However, we have previously shown that the fraction #6 mutagenicity in both the vapor and particle phases at Claremont, CA, can be accounted for by the measured ambient concentrations of 2-nitrodibenzopyranone at that same location and time (Helmig *et al.*, 1992b). Assuming that the 2-nitrodibenzopyranone (which elutes in HPLC fraction #6) in ambient air at Claremont arose from atmospheric reaction(s) of phenanthrene, then atmospheric transformations account for $\sim 50\%$ of the measured ambient air mutagenicity.

Clearly, there are uncertainties due to the fact that the ambient PAH concentrations were not measured together with the ambient atmospheric mutagenicity, leading to significant uncertainties in the atmospheric PAH burden during the mutagenicity measurement period. However, it is expected that to a reasonable approximation the PAH profile in the atmosphere should remain constant and that on a relative basis the PAH concentrations in the atmosphere should be fairly uniform as a function of location in the Los Angeles Basin, especially at mid-basin sites, relatively downwind from industrial source regions, such as Glendora and Claremont. Our data therefore indicate that the atmospheric reaction products of the gas-phase 2- to 4-ring PAH contribute $\sim 50\%$ of the ambient direct-acting mutagenicity, at least using our sampling methods and bioassay procedure with strain TA98.

It should be noted that we do not know all, or indeed many, of the reaction products responsible for the measured mutagenicity of the PAH photooxidations, especially the more polar products. Therefore, we cannot readily distinguish whether the mutagenic products collected from the chamber reactions will contribute to the gas-phase or particle-associated mutagenic activity of ambient air samples. If we assume that the fraction #3 and #4 activity is due largely to nitro-PAH, the mutagenicity from the two-ring PAH (naphthalene, 1- and 2-methyl-naphthalene, biphenyl, acenaphthene and acenaphthylene) will contribute to the gas-phase ambient mutagenicity, while the activity from the PAH with four or more rings will contribute

Table 26. Comparison of mutagenicities (revertants m^{-3}) for a simulated atmosphere with measured ambient mutagenicities.

	Mutagenicity in HPLC Fraction No.									
Source	1	2	3	4	5	6	7	8	9	Total
Chamber Photooxidations		1	1	84	10	12	11	3	1	123
Ambient										
Particle-phase			0.4	9.7	15	72	60	2.3	0.3	160
Vapor-phase	0.2		8.9	106	33	41	12	5.4	3.2	210
TOTAL	0.2		9.3	116	48	113	72	7.7	3.5	370

solely to the particle associated activity. The nitro-PAH products from fluorene, dibenzothiophene and phenanthrene are expected to be distributed between the gas and particle-phase, depending, in part, on the ambient temperatures involved. We have found the nitro-lactone products from phenanthrene to be distributed between the gas- and particle-phases, with up to 25% of the 2-nitrodibenzopyranone being collected on the PUF plug samples (see Table 1). It seems likely that the fraction #6 and #7 activity from pyrene will contribute mainly to particle-associated ambient mutagenicity.

It is also apparent that in the atmosphere several of the PAH investigated play only a minor role in the formation of atmospheric direct-acting mutagenicity, these being acenaphthene, acenaphthylene, biphenyl, retene, anthracene, benz[*a*]anthracene and chrysene. In contrast, naphthalene, 1- and 2-methylnaphthalene, fluorene, dibenzothiophene, phenanthrene, fluoranthene and pyrene are calculated to be important contributors to ambient mutagenicity through the formation of mutagenic reaction products in the atmosphere.

Another assessment of the role of the PAH studied in the formation of mutagenic products through their photooxidations can be obtained by ratioing the total number of revertants observed in the bioassay by the initial PAH concentrations in the photooxidations and the volumes sampled for mutagenicity assay. These data, in terms of revertants ppb^{-1} m^{-3}, are given in Table 27. The production of mutagenic products per ppb of PAH photooxidized, ranges over more than 3 orders of magnitude, and it is possible that these "mutagen formation potentials" can be used in assessing the impacts of, for example, changing the emissions profiles of combustion sources such as diesel exhaust.

Table 27. Measured direct-acting mutagenicities per ppb of PAH initially present in the chamber photooxidation.

PAH	Mutagenicity
	PAH concentration (revertants m^{-3} ppb^{-1})
Naphthalene	55
1-Methylnaphthalene	160
2-Methylnaphthalene	45
Acenaphthene	150
Acenaphthylene	340
Biphenyl	10
Fluorene	830
Dibenzothiophene	8300
Phenanthrene	1100
2-Methylphenanthrene	20-50
Anthracene	80 (40)[a]
Retene	160 (150)[a]
Fluoranthene	37000
Pyrene	18000
Benz(a)anthracene	0
Chrysene	~9000 (5000)[a]

[a]Subtracting chamber blank from the total activity.

SUMMARY AND CONCLUSIONS

Ambient air analyses demonstrated that the polar mutagenic reaction product of phenanthrene, 2-nitrodibenzopyranone, is a wide-spread contaminant in the environment. 2-Nitrodibenzopyranone was observed in all of the southern California ambient air filter and PUF plug samples analyzed, and 4-nitrodibenzopyranone was observed in the majority of these samples. Both isomers were observed in the SRM 1649 urban dust. 2-Nitrodibenzopyranone was quantified in all of the ambient air samples analyzed, at concentrations ranging between 0.04 and 0.8 ng m^{-3}.

The results obtained from this experimental study show that simulated atmospheric photooxidations of the gas-phase 2- to 4-ring PAH lead to mutagenic products. The mutagenic activities of the product mixture varies widely depending on the particular PAH, and also the mutagenicity profile varies from PAH to PAH, at least in terms of the polarity of the mutagenic products. In general, the PAH studied lead to either mutagenic nitro-PAH which elute, with the HPLC fractionation program used here, in fraction #3 or 4, or to more polar products including the nitro-PAH lactones. Our data, when compared with ambient air mutagenicity testing using the same sample collection, extraction, fractionation and bioassay testing procedures, allow ~50% of ambient air direct-acting mutagenicity, strain TA98, to be ascribed to PAH atmospheric transformation products formed in the atmosphere during transport from source to receptor. Our data also allow a ranking of the PAH studied to be made with respect to the number of revertants per unit PAH concentration ("mutagenicity formation potential"), with a range of over three orders of magnitude from biphenyl and benz[*a*]anthracene (the lowest) to fluoranthene (the highest). It is possible that this ranking can be used in assessments of emission changes brought about through emission control measures or the use of alternate fuels.

RECOMMENDATIONS

There are a number of areas requiring further work. These include:

- More detailed chemical analysis of the PAH reaction products and elucidation of the reaction mechanisms leading to these products in the atmosphere.

- An assessment of the contributions of the methylnitronaphthalenes to the semi-volatile ambient air mutagenicity.

- Ambient air measurements of the more polar mutagenic PAH reaction products such as the mutagenic nitro-PAH lactones formed from methylphenanthrenes and pyrene, and an assessment of the contributions of these polar mutagens to measured ambient air mutagenicity.

- Extension of this HPLC fractionation-bioassay approach to use human cell lines as the test organism rather than *Salmonella typhimurium*, in order to determine whether or not the present results obtained with the Ames bioassay system are applicable to more realistic mammalian test systems. Such studies could provide valuable information concerning the potential human health risks arising from emissions of gaseous 2- to 4-ring PAH into the atmosphere from combustion sources, including mobile sources.

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Glossary of terms, abbreviations, and symbols

ARB	California Air Resources Board
GC/FID	Gas chromatography with flame ionization detection
GC/MS	Gas chromatography/mass spectrometry
HPLC	High performance liquid chromatography
NIST	National Institute for Standards and Technology
NO	Nitric oxide
NO_2	Nitrogen dioxide
O_3	Ozone
o.d.	Outer diameter
OH	Hydroxyl radical
PAH	Polycylic aromatic hydrocarbons
PUF	Polyurethane foam
ppt	parts-per-trillion
SAPRC	Statewide Air Pollution Research Center
SCAQS	Southern California Air Quality Study
SIM	Selection ion monitoring
SRM	Standard Reference Material
TIC	Total ion chromatogram
U.S. EPA	United States Environmental Protection Agency